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Award Number: DAMD17-01-1-0281

TITLE: Enhancement of an Allogeneic GM-CSF-Secreting Breast Cancer Vaccine by Immunomodulatory Doses of Cyclophosphamide and Doxorubicin

PRINCIPAL INVESTIGATOR: Leisha A. Emens, M.D., Ph.D.
Elizabeth M. Jaffee, M.D.

CONTRACTING ORGANIZATION: The Johns Hopkins University School of Medicine
Baltimore, Maryland 21205

REPORT DATE: June 2003

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

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Dr. Katherine Moore _____

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Leisha A. Emens, M.D., Ph.D.
Elizabeth M. Jaffee, M.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
The Johns Hopkins University School of Medicine
Baltimore, Maryland 21205

E-Mail: emensle@jhmi.edu

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13. ABSTRACT (Maximum 200 Words)
Advanced breast cancer is managed with hormonal agents or conventional cytotoxic drugs, but intrinsic drug resistance ultimately causes treatment failure. We have applied the use of tumor cells genetically modified to secrete GM-CSF to the preclinical neu transgenic mouse model, characterized by spontaneous tumor development and pre-existing immune tolerance to HER-2/neu. Low doses of Cyclophosphamide and Doxorubicin in a specifically timed sequence with vaccine can augment the HER-2/neu-specific, vaccine-activated immune response in these mice, resulting in delayed tumor outgrowth compared to chemotherapy or vaccine alone. I have developed a GM-CSF-secreting breast cancer vaccine for human use, and designed a clinical trial to test it in patients with metastatic breast cancer. I have also designed a companion clinical study to govern the long-term follow-up of study participants. A number of regulatory approvals are in place including the FDA IND, the RAC, and the JHM-IRB-4. The immune monitoring assays are under development.

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Table of Contents

Annual Summary Report for Award Number DAMD17-01-1-0281: June 1, 2002 to May 31, 2003

PROPOSAL TITLE: An Allogeneic GM-CSF-secreting Breast Cancer Vaccine Administered Sequentially with Immunomodulatory Doses of Cyclophosphamide and Doxorubicin for the Treatment of Metastatic Breast Cancer

INTRODUCTION:

Advanced breast cancer is typically managed with hormonal agents or conventional cytotoxic drugs, but intrinsic drug resistance ultimately causes treatment failure. We have applied the use of tumor cells genetically modified to secrete GM-CSF to the preclinical *neu* transgenic mouse model, which is characterized by spontaneous tumor development (1) and pre-existing immune tolerance to HER-2/*neu* (2). Low doses of Cyclophosphamide and Doxorubicin in a specifically timed sequence with vaccine can augment the HER-2/*neu*-specific, vaccine-activated immune response in these mice, resulting in delayed tumor outgrowth compared to chemotherapy or vaccine alone (3). I have developed and characterized a GM-CSF-secreting breast cancer vaccine for clinical trials, and oversee its production in the Johns Hopkins GMP facility. I have also designed a Phase I vaccine safety and chemotherapy dose-finding trial evaluating timed sequential therapy with an allogeneic GM-CSF-secreting breast cancer vaccine and immune-modulating doses of Cyclophosphamide and Doxorubicin. The specific aims of the trial are to: 1) Evaluate the safety of the vaccination regimen; 2) Determine the doses of chemotherapy drugs that maximize the immune response by measuring immune responses to HER-2/*neu* as a sentinel marker of vaccine-activated immunity; 3) Assess the *in vivo* immune responses induced by the vaccination regimen by immunohistochemical analysis of vaccine site biopsies; and 4) Evaluate enrolled patients for time to disease progression.

BODY:

Research Training:

My clinically-oriented training in the management of breast cancer over the last year has been multi-faceted, consisting of patient care, clinical and research conferences at the Johns Hopkins School of Medicine, educational conferences sponsored by outside agencies, and research-oriented conferences focused on breast cancer. The details follow:

I have my own weekly continuity clinic focused on breast cancer patients beginning January 2002. I see two new patients each week for first and second opinions regarding hormonal therapy or chemotherapy for the treatment of primary or metastatic breast cancer. In this clinic, I have the opportunity to enroll patients on open clinical trials in the breast cancer research program at Johns Hopkins. I have responsibility for administering treatment, managing its complications, and providing follow-up care according to the current standards of care for breast cancer treatment.

I participate in the weekly multidisciplinary breast conference attended by the medical oncologists, the radiation oncologists, the breast surgical oncologists, and the providers in the high-risk clinic (Breast and Ovarian Surveillance Service (BOSS) at Johns Hopkins. This provides a forum for review of each new patient that is evaluated by the providers in the Breast Center at Johns Hopkins, and the development of a consensus for treatment that involves all of the staff. I also participate in a weekly meeting of the breast medical oncologists in the Breast Center. This meeting is designed to establish priorities for clinical research and manage the clinical trials portfolio, discuss the results of important new trials in breast cancer, and determine the most appropriate way to incorporate the new data into our breast cancer practices.

I attended four conferences focused specifically on breast cancer during the last year. The first was the Department of Defense Era of Hope Breast Cancer Meeting in September 25-28, 2002. I presented an abstract describing the development of the allogeneic, GM-CSF-secreting breast cancer vaccine at

that meeting. One was "Current Trends in Breast Cancer" sponsored by the Physician's Education Resource, held in Washington D.C. January 25, 2003. This meeting was designed as an update from the San Antonio Breast Cancer Symposium in December, 2002. The third was the UCI-Avon Breast Cancer Research and Care Symposium. I received an honorarium to attend this conference and present my research on the vaccine and clinical trial development. The fourth conference was the annual Avon research conference held at Emory University School of Medicine November 17-19, 2003. This conference gathers researchers from top breast cancer research programs around the country who receive Avon funding, and is a forum for review of their work. The material presented spans the spectrum of basic research, clinical research, and delivery of care.

My training in clinical research has consisted of the following:

I attend a weekly clinical research oversight meeting that reviews the status of all clinical trials in immunotherapy under development or open at Johns Hopkins. My mentor, Elizabeth Jaffee, directs this group.

I participate as a member of the oversight committee for the GMP facility at Johns Hopkins. This committee meets monthly. We review the status of current projects, and evaluate proposed projects for feasibility.

I am a co-investigator on a two breast cancer clinical trialsthat should launch within the next two months. One is "A Phase I Study of Weekly Taxotere (Docetaxel) and Gleevec (STI571, Imatinib mesylate, CGP-57148) in Locally Advanced or Metastatic Breast Cancer". The PI is Antonio Wolff, MD. The second is "A Pilot Study Assessing Patterns of Response or Resistance to Preoperative Docetaxel Chemotherapy in Women with Breast Cancer". The PI is Vered Stearns, M.D.

I have continued to develop my own clinical trial, which should launch in the next six to eight weeks. Additionally, I have developed a clinical protocol to govern the long-term follow-up of research subjects who receive the allogeneic, GM-CSF-secreting breast cancer vaccine. Long-term follow-up of research subjects who participate in clinical gene transfer trials is a federal mandate. This long-term follow-up protocol has been reviewed and approved by the Johns Hopkins IRB (JHM-IRB-4).

My training in basic research has consisted of the following:

I participate in the weekly meeting of the breast cancer research program. This meeting is designed to review the basic research ongoing in the laboratories of the members of the breast cancer research program at Johns Hopkins.

I participate in an immunology journal club and laboratory meeting, each weekly. These provide a forum for literature review and scientific critique.

I participate in a weekly symposium focused on immunotherapy, where speakers include experts from both within and outside the institution.

I continue to develop my own research project in the laboratory.

Statement of Work:

Task 1. Recruit 27 patients with Stage IV breast cancer (months 1-24):

Obtain Institutional Review Board approval from The Johns Hopkins Hospital and Department of Defense. Institutional review board approval has been obtained from The Johns Hopkins IRB (JHM-IRB-4). Approval was originally granted 5/22/01. The protocol and consents were re-reviewed, and the protocol renewal was re-approved and renewed on 3/18/02, and again on April 7, 2004. Initial DOD review of the protocol is complete, and the memorandum was received in early June 2002. I have addressed the points raised in the memorandum, and will re-submit the material to the DOD IRB by June 30, 2003, as I have now successful undergone FDA review of the investigational new drug (IND) application (BB-IND#11019), and the RAC submission (protocol #0304-578). Also, I have prepared

clinical standard operating procedures to meet current standards of Good Clinical Practice, and have a data and safety monitoring plan in place.

Submit and obtain approval for an investigational new drug. We have completed production of two master cell banks (MCBs) of vaccine cells, and they have passed regulatory testing. We have also completed the production and release testing on two pilot clinical lots of vaccine cells, and full-scale clinical lots are currently in production in the Cell Processing and Gene Therapy Facility at Johns Hopkins. Additionally, I was awarded a contract through the Rapid Access to Investigational Drugs (RAID) program sponsored by the National Cancer Institute. This contract is an agreement for the RAID program to formulate and vial the clinical grade peptides for the testing of delayed type hypersensitivity in the clinical trial. All three peptides have been formulated and vialed by RAID, and the formulated peptides are currently undergoing regulatory testing. I expect released material to be available within the month.

I also prepared and submitted the IND to the FDA. The review team had several concerns, which I responded to. After submission on April 9, 2003, we were given verbal clearance to begin the trial on May 9, 2003. I also prepared and submitted the RAC document (Appendix M), and received RAC approval on May 12, 2003.

With these federal regulatory approvals in place, I have now modified the clinical protocols to meet the concerns of the FDA, the DOD IRB, and the JHM-IRB-4. I anticipate submitting these documents for final IRB review and approval for trial accrual within the week. I also anticipate submitting the application to the Institutional Biosafety Committee (IBC) within the week.

Tasks 1c and 1d require all regulatory approvals to be in place, including the FDA, NIH/OBA/RAC, DOD, and The Johns Hopkins JCCI. The trial should launch within the next two months.

Task 2. Evaluate the safety of the vaccination regimen (months 5-36).

All components of task 2 require all regulatory approvals to be in place, including the FDA, NIH/OBA/RAC, DOD, and The Johns Hopkins JCCI. The trial should launch within the next two months.

Task 3. Identify the doses of chemotherapy drugs that maximize vaccine-activated immunity by analyzing immunity to HER-2/*neu* as a surrogate for the anti-tumor immune response (months 1-36).

a) Develop ELISA and ELISPOT assays for measuring antibody and T cells specific for HER-2/*neu* in patient sera. I have established a collaboration with Dr. Mary L. Disis, who is internationally known for her work in HER-2/*neu* peptide-based vaccines. She and her colleagues have developed and standardized a human HER-2/*neu* ELISA method for determining antibody titers following therapy with their peptide vaccines (4, 5). She has agreed to determine the titers of developing HER-2/*neu*-specific antibody using their assay. Additionally, we have initiated the development of the ELISPOT assay for HER-2/*neu*-specific $CD4^+$ T cells by performing initial studies with interferon-γ-secreting CD8+ T cells specific for the influenza M1 protein. This assay has been used to analyze immune responses in a clinical trial of a similar GM-CSF-secreting vaccine for pancreatic cancer. We are using this as a platform to further optimize the assay for the detection of fewer cells (improved sensitivity), and adapt it for the detection of cytokine-secreting $CD4^+$ T cells using antibodies specific for both interferon-γ and interleukin-4.

Tasks 3b, 3c, and 3d require all regulatory approvals to be in place, including the FDA, NIH/OBA/RAC, DOD, and the JHM-IRB-4. The trial should launch within the next six months.

Task 4. Assess clinical status and time to disease progression (months 5-36).

This task requires all regulatory approvals to be in place, including the FDA, NIH/OBA/RAC, DOD, and the JHM-IRB-4. The trial should launch within the next six months.

KEY RESEARCH ACCOMPLISHMENTS:

1. Completed genetic modification and characterization of three allogeneic breast cancer cell lines for vaccine development (see Tables 1 and 2 in appendices).
2. Completed production and certification of two master cell banks of genetically-modified allogeneic breast cancer cells from which to derive clinical grade vaccine cells.
3. Successfully completed production and certification of pilot scale clinical lots from each of the two master cell banks. Full scale clinical lots are currently in production.
4. Obtained funding through the RAID program of the Developmental Therapeutics Program at the NCI to complete formulation, vialing, and release testing of the peptides for clinical use.
5. Completed formulation and vialing of clinical grade peptides for delayed type hypersensitivity testing. These peptides are now in regulatory testing, and should be released within the next few weeks.
6. Obtained and maintained IRB approvals for the protocol and consent forms for the proposed trial.
7. Developed case report forms, clinical standard operating procedures and a data safety and monitoring plan to govern the conduct of the trial according to Good Clinical Practice.
8. Completed and submitted an investigation new drug (IND) application to the FDA. I am the sponsor of this IND (BB-IND#11019). It has been reviewed by them, and modified according to their recommendations, with a verbal approval granted May 9, 2003.
9. Completed and submitted the RAC document (Appendix M). Received approval with no concerns May 9, 2003.
10. Established a collaboration with Dr. Mary L. Disis to determine titers of HER-2/*neu* antibody in patient sera for the trial.
11. Initiated initial development of ELISPOT assays for trial-related immune monitoring of HER-2/*neu*-specific immune responses.

REPORTABLE OUTCOMES:

Regulatory Approvals:

1. FDA IND BB-IND#11019
2. RAC protocol #0304-578
3. IRB approval, J0085: A Phase I Vaccine Safety and Chemotherapy Dose-Finding Trial of an Allogeneic GM-CSF-secreting Breast Cancer Vaccine in a Specifically Timed Sequence with Cyclophosphamide and Doxorubicin.
4. IRB approval, J0320: Long-Term Follow-Up of Research Subjects who Received the GM-CSF-secreting Allogeneic Breast Cancer Vaccine

Publications:

1. **Emens, LA**, Biedrzycki, B, Davidson N, Davis-Sproul, J, Fetting, J, Masayesva, S, Onners, B, Piantadosi, S, Reilly, RT, Wolff, A, and Jaffee, EM. 2003. A Phase I Vaccine Safety and Chemotherapy Dose-Finding Trial of an Allogeneic GM-CSF-secreting Breast Cancer Vaccine Given in a Specifically Timed Sequence with Immunomodulatory Doses of Cyclophosphamide and Doxorubicin. Human Gene Therapy, (clinical protocol) *in press*.
2. Wolpoe, ME, Lutz, ER, Ercolini, AC, Murata, S, Ivie, S, Garrett, ES, **Emens, LA**, Jaffee, EM, and Reilly, RT. 2003. *Neu*-specific Monoclonal Antibodies Collaborate with HER-2/*neu* Specific GM-CSF-Secreting Whole-Cell Vaccination to Augment $CD8^+$ T Cell Effector Function and Tumor-Free Survival in HER-2/*neu* Transgenic Mice, *in press*.
3. **Emens, LA** and Jaffee, EM. 2003. Cancer Vaccines: An Old Idea Comes of Age. Cancer Biology and Therapy, 2 (suppl 1): 131-138.

4. **Emens, LA**. 2003. A New Twist on Autologous Cancer Vaccines. Cancer Biology and Therapy, 2:161-163.
5. **Emens, LA** and Jaffee, EM. 2003. Gene-Modified Tumor Cell Vaccines. In: Handbook of Cancer Vaccines, Humana Press, *in press*.
6. **Emens, LA**, Reilly RT, and Jaffee, EM. 2003. Cancer Vaccines in Combination with Multimodality Therapy. Cancer Treatment and Research, *in press*.
7. **Emens, LA** and Jaffee, EM. 2003. Towards a Breast Cancer Vaccine: Work in Progress. Oncology, *in press*.
8. Ercolini, AM, Ladle, B, Armstrong, TD, **Emens, LA**, Machiels, J-PH, Reilly, RT, and Jaffee, EM. 2003. Peripheral Anergy of High Avidity $CD8^+$ T Cells for the Immunodominant Rat HER-2/*neu* Epitope Explains the Mechanism of $CD8^+$ T Cell Tolerance in HER-2/*neu* Transgenic Mice. Nature Medicine, submitted.

Abstracts:

1. **Emens, LA**, Davis-Sproul, J, Thomas, AM, Reilly RT, Davidson, NE, and Jaffee, EM. 2002. Development of a Lethally-Irradiated GM-CSF-secreting Allogeneic Breast Cancer Vaccine for Use in Clinical Trials. Era of Hope Department of Defense Breast Cancer Research Program Meeting.
2. Wolpoe, M, Lutz, E, Ercolini, A, Greene, M, **Emens, L**, Jaffee, E, and Reilly, RT. 2002. Combined Passive (Monoclonal Antibody Infusion) and Active (Whole-Cell Vaccination) Immunotherapy is More Effective than Either Modality Alone in the Eradication of HER-2/*neu*-Expressing Mammary Tumors. Tenth SPORE Investigator's Workshop.
3. Ercolini, AM, Ladle, B, Armstrong, TD, Machiels, J-PH, Emens, LA, Reilly, RT, and Jaffee, EM. 2003. Reversal of $CD8^+$ Peripheral Tolerance in the HER-2/*neu* Transgenic Mice by Deletion of $CD4^+CD25^+$ Regulatory T Cells. Basic Mechanisms of Antitumor Immunity, 028:52; Keystone, CO.
4. Ercolini, AM, Armstrong, T, Ladle, B, Machiels, J-PH, Lei, R, **Emens, LA**, Reilly, RT, and Jaffee, EM. 2003. An Alternate, Lower-Affinity *Neu*-Specific T Cell Repertoire in HER-2/*neu* Transgenic Mice Relative to the Parental Strain May Explain *Neu*-Specific Tolerance to *Neu*-Expressing Tumors. Basic Mechanisms of Antitumor Immunity, **318**: 100; Keystone, CO.
5. Ladle, BH, Manning, EM, **Emens, LA**, Ercolini, AM, Machiels, J-PH, and Jaffee, EM. 2003. Reversal of $CD8^+$ Peripheral Tolerance in the HER-2/*neu* Transgenic Mice by Deletion of $CD4^+CD25^+$ Regulatory T Cells. Basic Mechanisms of Antitumor Immunity, **333**: 104; Keystone, CO.
6. Wolpoe, M, Lutz, E, Ercolini, A, Ivie, S, **Emens, L**, Jaffee, E, and Reilly RT. 2003. Combined Passive (Monoclonal Antibody Infusion) and Active (Whole-Cell Vaccination) Immunotherapy is More Effective than Either Modality Alone in the Eradication of HER-2/*neu*-expressing Mammary Tumors. Basic Mechanisms of Antitumor Immunity, **170**: 85; Keystone, CO.

Presentations:

1. "Chemotherapy: Friend or Foe to Cancer Vaccines?" RAID/SAIC-Frederick, Incorporated, Dev Therapeutics Program, NCI-Frederick, Frederick, MD September 2002.
2. "Timed Sequential Therapy with a Breast Vaccine and Immunomodulatory Doses of Chemotherapy" Third Annual University of California, Irvine/Avon Products Foundation Breast Cancer Research and Care Symposium, UCI University Club, Irvine, CA October 21, 2002

Grants and Contracts:

Awarded

1. Career Development Award, P50 CA 88843 NIH Breast Cancer SPORE (PI: Nancy E. Davidson, M.D.): "The Role of $CD4^+$ $CD25^+$ T Cells in Maintaining Immune Tolerance in *Neu* Transgenic Mice." Project Dates: 01/01/02-12/31/04. Total costs: $50,000/year.

2. Principal Investigator, RAID VIII, #178 (Rapid Access to Intervention Development) Program, Division of Cancer Treatment and Diagnosis, Developmental Therapeutics Program, National Cancer Institute: "Chemoimmunotherapy for Metastatic Breast Cancer Treatment". Priority Score: 1.98 (scale from 1 to 5: 1=outstanding and 5=defer). Total estimated costs: $195,000.
3. Principal Investigator, Maryland Cigarette Restitution Fund, "Timed Sequential Therapy with Cyclophosphamide, Doxorubicin, and a Breast Cancer Vaccine." Project Dates: 07/01/02-06/30/04. Total direct costs: $100,000.
4. Principal Investigator, Johns Hopkins University School of Medicine Clinician-Scientist Award, "Chemoimmunotherapy for Breast Cancer Treatment." Project Dates: 01/01/03-12/31/04. Total direct costs: $52,374.
5. Co-Investigator (5%), AVON Foundation (Principal Investigator: Elizabeth M. Jaffee, M.D.): "The Baltimore/Seattle Breast Cancer Immunotherapy Collaborative." Project Dates: 10/01/02-09/30/05. Total direct costs: $1,230,397.

Pending

1. Principal Investigator, NIH/NCI K23 Mentored Patient-Oriented Research Career Development Award (1 K23 CA098498-01), "Chemoimmunotherapy for Breast Cancer Treatment." Project Dates: 12/01/02-11/30/07. Total direct costs: $707,486
2. Principal Investigator, Wendy Will Case Cancer Fund: "Unveiling Potentially Novel Mechanisms of Anti-Tumor Synergy Between Targeted Angiocidal and Immune-Based Therapies." Project Dates: 07/01/03-06/30/04. Total direct costs: $50,000.

Employment:

Appointed Assistant Professor of Oncology, Johns Hopkins University School of Medicine, 11/01/01.

CONCLUSIONS:

I have made significant progress in the development, characterization, and production of the vaccine required for the proposed clinical study. I have also designed an approved clinical trial, and have made significant progress in obtaining relevant regulatory approvals. I have successfully obtained additional research funding that will support the trial in addition to that already available (from the RAID program, the Maryland Cigarette Restitution Fund, and the Johns Hopkins Clinician Scientist Award). I have also made progress in developing the reagents and assays that will be required for the immune monitoring studies. I continue to actively develop a clinical breast cancer practice that will support the continued development of my clinical skills in breast cancer, and will serve as a referral base for the trial. Finally, I have obtained a faculty position at Johns Hopkins.

REFERENCES:

1. Guy, CT, Webster, MA, Schaller, M, Parsons, TJ, Cardiff, RD, and Muller, WJ. Expression of the *neu* protooncogene in the mammary epithelium of transgenic mice induces metastatic disease. Proceedings of the National Academy of Sciences, USA. 89: 10578-10582, 1992.
2. Reilly, RT, Gottleib, MBC, Ercolini, AM, Machiels, J-PH, Kane, CE, Okoye, FI, Muller, WJ, Dixon, KH, and Jaffee, EM. HER-2/*neu* is a tumor rejection target in tolerized HER-2/*neu* transgenic mice. Cancer Research. 60: 3569-3576, 2000.
3. Machiels, J-PH, Reilly, RT, Emens, LA, Ercolini, AM, Lei, RY, Weintraub, D, Okoye, FI, and Jaffee, EM. Cyclophosphamide, Doxorubicin, and Paclitaxel enhance the anti-tumor immune response of GM-CSF-secreting whole-cell vaccines in HER-2/*neu* tolerized mice. Cancer Research. 61: 3689-3697, 2001.
4. Disis, ML, Pupa, SM, Gralow, JR, Dittadi, R, Menard, S, and Cheever, MA. High-titer HER-2/*neu* protein-specific antibody can be detected in patients with early-stage breast cancer. Journal of Clinical Oncology. 15: 3363-3367, 1997.

5. Disis, ML, Grabstein, KH, Sleath, PR, and Cheever, MA. Generation of immunity to the HER-2/*neu* oncogenic protein in patients with breast and ovarian cancer using a peptide-based vaccine. Clinical Cancer Research. 5: 1289-1297, 1999.

APPENDICES:

1. Johns Hopkins JHM-IRB-4 approval, J0085
2. Johns Hopkins JHM-IRB-4 approval, J0320
3. BB-IND#11019
4. RAC protocol #0304-578
5. journal articles and abstracts

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MEDICINE

Institutional Review Boards

Office of Human Subjects Research
East Baltimore Campus (JHM IRBs 1 – 4)
Turner Building, Room 36
720 Rutland Avenue
Baltimore, MD 21205-2196
(410) 955-3008 FAX (410) 955-4367

Bayview Campus (JHM IRB 5)
Asthma & Allergy Building, Rm. 3B74
5501 Hopkins Bayview Circle
Baltimore, MD 21224
(410) 550-1853 FAX (410) 550-0877

JHM-IRB 4

CONTINUING REVIEW APPROVAL NOTICE

TO : Leisha Emens
Assistant Professor, Oncology
4M07The Bunting Blaustein Can

FROM: Hayden Braine, M.D.
Chairman - JHM-IRB 4

DATE: June 11, 2003

RE : **Application NO:01-01-25-02, entitled, A Phase I Vaccine Safety and Chemotherapy Dose Finding Trial of an Allogeneic GM-CSF Secreting Breast Cancer Vaccine Given in a Specifically Timed Sequence With Immunomodulatory Doses of Cyclophosphamide and Doxorubicin (with Deborah Armstrong, Barbara Biedrzycki, Nancy Davidson, Carol Declue, Michele Donehower, Deborah Epstein, John Fetting, Jean-Pascal Machials, Steve Piantadosi, Todd Reilly, Antonio Wolff, Elizabeth Jaffee)**

I am pleased to inform you that at the convened meeting of **04/07/2003** the JHM-IRB 4 voted to approve the above-referenced protocol. Re-approval of the protocol and the consent form(s) is for the period of **04/07/2003 to 04/07/2004**. **The consent forms will not be released, no recruitment until IND# is on file.** As principal investigator of the project, you are responsible for fulfilling the following requirements of approval:

1) The co-investigators listed on the application should be kept informed of the status of the project.

2) Changes, amendments, and addenda to the protocol or the consent form must be submitted to the JHM-IRB 4 for re-review and approval prior to the activation of the changes. The application number assigned to the project should be cited in any correspondence.

3) Adverse events should be reported to the JHM-IRB 4 promptly. New information that becomes available which could change the risk:benefit ratio must be submitted promptly for JHM-IRB 4 review. The JHM-IRB 4 and outside agencies must review the information to determine if the protocol should be modified, discontinued, or continued as originally approved

4) Only consent forms with a valid approval stamp may be presented to subjects. All consent forms signed by subjects enrolled in the study should be retained on file. The JHM-IRB 4 conducts periodic audits of protocol records, and consent documentation is part of such audits.

5) Federal regulations require review of an approved study not less than once per 12-month period. Therefore, a continuing review application must be submitted to the JHM-IRB 4 office six weeks prior to the above expiration date of 04/07/2004. This will allow sufficient time for review of the application to be completed prior to the anniversary of the original approval date. Failure to submit a continuing review application in a timely fashion will result in termination of the study, at which point new subjects may not be enrolled and currently enrolled subjects must be taken off of the study.

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East Baltimore Campus (JHM IRBs 1 – 4)
Turner Building, Room 36
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Baltimore, MD 21205-2196
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Bayview Campus (JHM IRB 5)
Asthma & Allergy Building, Rm. 3B74
5501 Hopkins Bayview Circle
Baltimore, MD 21224
(410) 550-1853 FAX (410) 550-0877

May 15, 2003

PENDING NOTICE: **05/12/2003**
New Protocol Application

TO : Leisha Emens
Assistant Professor, Oncology
4M07The Bunting Blaustein Can

FROM: Hayden Braine, M.D.
Chairman - JHM-IRB 4

RE : **Application NO:03-04-17-02, entitled, Long-Term Follow-Up of Research Subjects who Received the GM-CSF-secreting Allogeneic Breast Cancer Vaccine (with Barbara Biedrzycki, Irena Tartakovsky, Beth Onners)**

The **JHM-IRB 4** reviewed your request for the above-referenced during a convened meeting held on **05/12/2003**. The study was granted **conditional approval.** Please resolve the following issues:

1. The Committee is concerned that testing required by this protocol. It could be obtained either by the clinical physician or by the investigators. If it is by the investigators, it is currently the intention that testing be paid for by the subjects' insurance. The Committee believes that this is inappropriate, as the subjects may have financial risks through co-pays or the lack of insurance coverage. Further, the patient encumbers the risk if the referring physician does not order appropriate testing. Therefore, approval is contingent upon obtaining the information from the referring physician or an alternate source of funding should be identified for payment for testing.

2. Consent form changes are requested, as indicated on the attached mark-up.

Upon receipt of your response, the review will continue. Please note that you will not receive further written reminders from the IRB that you need to respond to the above stated issues. **IF A RESPONSE IS NOT RECEIVED WITHIN 2 MONTHS OF THE DATE OF THIS LETTER, YOUR REQUEST WILL BE ADMINISTRATIVELY WITHDRAWN.**

Responses may be sent by e-mail to ohsceb@jhmi.edu or faxed to the **JHM-IRB 4** office at **410-955-4367**. To facilitate our review, please restate the committee's requests/questions in your response. In addition, **Bold** or underline any changes in respective documents (protocol application, consent forms, radiation forms, drug data sheets, etc.) and provide a clean copy.

Thank you.

HB:croberts

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville MD 20857

Our Reference: BB-IND 11019

APR 15 2003

Leisha A. Emens, M.D., Ph.D.
1650 Orleans Street Room 4M90
Bunting Blaustein Cancer Research
Building
Baltimore, MD 21231-1000

Dear Dr. Emens:

The Center for Biologics Evaluation and Research has received your **Investigational New Drug Application (IND)**. The following product name and BB-IND number have been assigned to this application. They serve only to identify it and do not imply that this Center either endorses or does not endorse your application.

BB-IND #: 11019

SPONSOR: Leisha A. Emens, M.D., Ph.D.

PRODUCT NAME: Allogeneic Breast Cancer Cell Lines Transfected with DNA Plasmid Vector PCDNA1-neo, Invitrogen) Expressing Granulocyte-Macrophage Colony-Stimulating Factor; with or with out Chemotherapy

DATE OF SUBMISSION: April 11, 2003

DATE OF RECEIPT: April 11, 2003

This BB-IND number should be used to identify all future correspondence and submissions, as well as telephone inquiries concerning this IND. Please provide an **original and two copies of every submission to this file**. Please include three originals of all illustrations which do not reproduce well.

It is understood that studies in humans will not be initiated until 30 days after the date of receipt shown above. If this office notifies you, verbally or in writing, of serious deficiencies that require correction before human studies can begin, it is understood that you will continue to withhold such studies until you are notified that the material you have submitted to correct the deficiencies is satisfactory. If such a clinical hold is placed on this file, you will be notified in writing of the reasons for placing the IND on hold.

A copy of this Acknowledgement Letter and the name, address and telephone number of the clinical investigator(s) as provided on Form FDA 1572 will be forwarded to the National

Institutes of Health (NIH) Office of Biotechnology Activities (OBA). The information provided will be used internally by NIH OBA solely to monitor compliance with the NIH Guidelines for Research Involving Recombinant DNA Molecules (http://www.nih.gov/od/oba/). NIH's Guidelines describe submission and reporting requirements that are applicable to 1) research conducted at or sponsored by an institution that receives *any* support for recombinant DNA research from the NIH, including research performed directly by NIH, and 2) clinical researchers that collaborate or contract with another institution that developed recombinant DNA materials with NIH funds. **Should your research fall into this category, please be aware the clinical trial proposal must be submitted to OBA and that no patients may be enrolled until NIH Recombinant DNA Advisory Committee (RAC) review is completed.** Sponsors of human gene therapy protocols subject to the NIH Guidelines are also required to submit to NIH OBA all new clinical trial sites, all new protocols and protocol changes (including those initiated by the sponsor and those resulting from either FDA or the RAC review), and all serious adverse events. Please refer to the November 5, 1999, Dear Gene Therapy IND Sponsor/Principal Investigator Letter (http://www.fda.gov/cber/ltr/gt110599.htm) and the Federal Register notice of amendments to the NIH Guidelines (65 FR 60328, http://www4.od.nih.gov/oba/1010frnotice.pdf). Reports submitted to NIH OBA shall be sent to the Office of Biotechnology Activities, National Institutes of Health/MSC 7010, 6000 Executive Boulevard, Suite 302, Bethesda, Maryland 20892-7010. Contact NIH OBA at (301) 496-9838 for further information.

Please refer to the FDA's March 6, 2000 letter to all gene therapy sponsors (available at http://www.fda.gov/cber/ltr/gt030600.htm):

a) **If you have not provided in this IND the information requested in that letter, it should be promptly submitted for review by FDA prior to initiation of studies. Failure to submit the information in a timely fashion will result in a clinical hold.**

b) Also submit yearly manufacturing updates addressing all information requested in items 1 through 4 of the March 6, 2000 letter. Please also affirm, on a yearly basis, that manufacturing QA and QC and clinical trial oversight and monitoring have been conducted per the plans submitted to FDA, and submit modifications or updates to those plans as appropriate. For administrative convenience, we request that you provide this information in your annual reports.

Because of concerns regarding potential long-term adverse events, **a long-term clinical monitoring program must be in effect for studies under this IND**. Your IND should provide sufficient details to indicate that monitoring is consistent with recent advice and recommendations provided to the FDA by the Biologic Response Modifiers Advisory Committee (BRMAC). In general, BRMAC has recommended that long-term follow-up should extend over a period of 15 years and include all gene transfer studies. Long-term follow-up should focus on the collection of clinical information pertaining to de novo cancer, neurologic, autoimmune, and hematologic disorders. In addition, unexpected medical problems including

information on hospitalizations and medications should be collected. Please refer to the BRMAC October 24, 2001 briefing material and meeting transcripts for further details (http://www.fda.gov/ohrms/dockets/ac/cber01.htm). **If provisions for long-term follow-up were not included in your IND submission, please promptly submit the information for review by FDA prior to initiation of studies. Failure to submit the information in a timely fashion will result in a clinical hold.**

You are responsible for compliance with applicable portions of the Public Health Service Act, the Federal Food, Drug, and Cosmetic Act, and the Code of Federal Regulations (CFR). A copy of 21 CFR Part 312, pertaining to INDs, is enclosed. Copies of other pertinent regulations are available from this Center upon request. The following points regarding obligations of an IND sponsor are included for your information only, and are not intended to be comprehensive.

Progress reports are required at intervals not exceeding one year and are due within 60 days of the anniversary of the date that the IND went into effect [21 CFR 312.33]. These progress reports should also include data from your long-term follow-up of patients.

Any unexpected fatal or immediately life-threatening reaction associated with use of this product must be reported to this Division by telephone or facsimile transmission no later than seven calendar days after initial receipt of the information. All serious, unexpected adverse experiences, as well as results from animal studies that suggest significant clinical risk, must be reported, in writing, to this Division and to all investigators within fifteen calendar days after initial receipt of this information [21 CFR 312.32]. **Please be advised that for all protocols under this IND, you are required to gather such information and continue safety reporting to this IND, for as long as your protocol(s) or IND indicate (21 CFR 312.60) regardless of whether the study is discontinued for any reason or whether the IND is inactivated.**

Charging for an investigational product in a clinical trial under an IND is not permitted without the prior written approval of the FDA.

Prior to use of each new lot of the investigational biologic in clinical trials, please submit the lot number, the results of all tests performed on the lot, and the specifications when established (i.e., the range of acceptable results).

If not included in your submission, please provide copies of the consent forms for each clinical study. A copy of the requirements for and elements of informed consent are enclosed. Also, please provide documentation of the institutional review board approval(s) for each clinical study.

All laboratory or animal studies intended to support the safety of this product should be conducted in compliance with the regulations for "Good Laboratory Practice for Nonclinical Laboratory Studies" (21 CFR Part 58, copies available upon request). If such studies have not been conducted in compliance with these regulations, please provide a statement describing in

detail all differences between the practices used and those required in the regulations.

Item 7a of form FDA 1571 requests that either an "environmental assessment," or a "claim for categorical exclusion" from the requirements for environmental assessment, be included in the IND. If you did not include a response to this item with your application, please submit one. See the enclosed information sheet for additional information on how these requirements may be addressed.
Telephone inquiries concerning this IND should be made directly to me at (301) 827-5102. Correspondence regarding this file should be addressed as follows:

Center for Biologics Evaluation and Research
Attn: Office of Cellular, Tissue, and Gene Therapies
HFM-99, Room 200N
1401 Rockville Pike
Rockville, MD 20852-1448

If we have any comments after we have reviewed this submission, we will contact you.

Sincerely yours,

Lori Tull
Regulatory Project Manager
Regulatory Management Staff
Office of Cellular, Tissue,
and Gene Therapies
Center for Biologics
Evaluation and Research

Enclosures (3): 21 CFR Part 312
21 CFR 50.20, 50.25
Information sheet on 21 CFR 25.24

Information Sheet for a Claim of Categorical Exclusion for an IND Under 21 CFR 25.24

For those wastes generated in the production and use of the product which will be controlled, please include documentation that such waste storage or disposal is in compliance with federal, state and local requirements for hazardous waste production. As an alternative, identify any generally recognized, scientifically sound control procedures which have been implemented to reduce the likelihood of inadvertent release of potentially toxic materials into the environment (e.g., compliance with the NIH Guidelines for Research Involving Recombinant DNA Molecules [51 FR 16958 (1986)] and/or compliance with the EPA Effluent Guidelines and Standards for Pharmaceutical Manufacturing [40 CFR 439]). If these alternatives are not applicable, a description of the control procedures actually used to prevent waste from entering the environment should be submitted.

For those wastes generated in the production and use of the product which will not be controlled, please list the potentially toxic waste compounds, including the quantities and concentrations which may be expected to enter the environment from both productions of the product and from the intended clinical studies, and briefly describe the immediate environment into which such release will occur. Further, provide the appropriate references or experimental data from which it may be reasonably concluded that such release is non-toxic.

If the waste to be generated during the production and proposed investigational use of this product is either not controlled or is not reasonably expected to be non-toxic in the environment to which it will be released, please submit an environmental assessment using the format described in 21 CFR 25.31.

If actions under proposed amendments to this IND substantially alter the quantity, quality or conditions of waste release in such a way as to alter the basis for either a claim of categorical exclusion or an environmental assessment, then such amendments should be supported by the appropriate data for a claim of categorical exclusion or an amended environmental assessment for wastes generated under the proposed amendments to this IND.

An investigator sponsored IND for which no additional product manufacturing is intended will ordinarily have addressed these environmental issues by incorporating the manufacturer's IND or MF by cross reference. However, if the use of the product during clinical investigation is expected to result in the uncontrolled release of toxic materials into the environment then an environmental assessment should be submitted.

3/30/94

§ 50.20 General requirements for informed consent.

Except as provided in § 50.23, no investigator may involve a human being as a subject in research covered by these regulations unless the investigator has obtained the legally effective informed consent of the subject or the subject's legally authorized representative. An investigator shall seek such consent only under circumstances that provide the prospective subject or the representative sufficient opportunity to consider whether or not to participate and that minimize the possibility of coercion or undue influence. The information that is given to the subject or the representative shall be in language understandable to the subject or the representative. No informed consent, whether oral or written, may include any exculpatory language through which the subject or the representative is made to waive or appear to waive any of the subject's legal rights, or releases or appears to release the investigator, the sponsor, the institution, or its agents from liability for negligence.

§ 50.25 Elements of informed consent.

(a) *Basic elements of informed consent.* In seeking informed consent, the following information shall be provided to each subject:

(1) A statement that the study involves research, an explanation of the purposes of the research and the expected duration of the subject's participation, a description of the procedures to be followed, and identification of any procedures which are experimental.

(2) A description of any reasonably foreseeable risks or discomforts to the subject.

(3) A description of any benefits to the subject or to others which may reasonably be expected from the research.

(4) A disclosure of appropriate alternative procedures or courses of treatment, if any, that might be advantageous to the subject.

(5) A statement describing the extent, if any, to which confidentiality of records identifying the subject will be maintained and that notes the possibility that the Food and Drug Administration may inspect the records.

(6) For research involving more than minimal risk, an explanation as to whether any compensation and an explanation as to whether any medical treatments are available if injury occurs and, if so, what they consist of, or where further information may be obtained.

(7) An explanation of whom to contact for answers to pertinent questions about the research and research subjects' rights, and whom to contact in the event of a research-related injury to the subject.

(8) A statement that participation is voluntary, that refusal to participate will involve no penalty or loss of benefits to which the subject is otherwise entitled, and that the subject may discontinue participation at any time without penalty or loss of benefits to which the subject is otherwise entitled.

(b) *Additional elements of informed consent.* When appropriate, one or more of the following elements of information shall also be provided to each subject:

(1) A statement that the particular treatment or procedure may involve risks to the subject (or to the embryo or fetus, if the subject is or may become pregnant) which are currently unforeseeable.

(2) Anticipated circumstances under which the subject's participation may be terminated by the investigator without regard to the subject's consent.

(3) Any additional costs to the subject that may result from participation in the research.

(4) The consequences of a subject's decision to withdraw from the research and procedures for orderly termination of participation by the subject.

(5) A statement that significant new findings developed during the course of the research which may relate to the subject's willingness to continue participation will be provided to the subject.

(6) The approximate number of subjects involved in the study.

(c) The informed consent requirements in these regulations are not intended to preempt any applicable Federal, State, or local laws which require additional information to be disclosed for informed consent to be legally effective.

(d) Nothing in these regulations is intended to limit the authority of a physician to provide emergency medical care to the extent the physician is permitted to do so under applicable Federal, State, or local law.

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Office of Biotechnology Activities
National Institutes of Health
6705 Rockledge Drive
Suite 750, MSC 7985
Bethesda, MD 20892-7985
(301) 496-9838 (Phone)
(301) 496-9839 (Fax)
http://www4.nih.gov/oba/

May 12, 2003

Leisha A. Emens, M.D., Ph.D.
Assistant Professor of Oncology
The Sidney Kimmel Comprehensive Cancer Center
Johns Hopkins University
1650 Orleans St., Room 4M-90
Baltimore, MD, 21231

RE: Protocol #0304-578 entitled: *A phase I vaccine safety and chemotherapy dose-finding trial of allogeneic GM-CSF-secreting breast cancer vaccine given in a specifically timed sequence with immunomodulatory doses of cyclophosphamide and doxorubicin*

Dear Dr. Emens:

I am writing to notify you of the outcome of the initial review of your submission by members of the National Institutes of Health (NIH) Recombinant DNA Advisory Committee (RAC).

As you know, in accordance with Appendix M of the *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)*, members of the RAC carried out an initial review of your submission to assess whether it raised any significant issues that warrant further review and discussion by the RAC in a public session. They were provided a copy of the entire protocol submission as well as a brief summary of the submission's main features. After review of this material and other relevant information, it was determined that your submission does not require an in-depth RAC review and public discussion.

As you know, during the initial review process, RAC members may request additional information or clarification about the submission. They also may have specific comments or suggestions about the protocol design, informed consent document, or other matters. These questions and comments were conveyed to you and you were provided an opportunity to address them. Since these comments represent the considered perspectives of individual members and do not constitute a consensus of the RAC, you are encouraged, but not required to, consider them further. This correspondence becomes part of the public record of the protocol submission and is available upon request to the investigator(s), sponsor (if applicable), Institutional Biosafety Committee (IBC) and Institutional Review Board (IRB) as well as members of the public. Requests should be directed to the NIH Office of Biotechnology Activities (OBA) via email (oba@od.nih.gov) or via telephone (301-496-9838). The NIH OBA protocol number should be included in any request.

The Principal Investigator and the institution are responsible for ensuring that no research participants are enrolled in the protocol until IBC approval, IRB approval and all applicable regulatory authorizations have been obtained. Please be mindful that even though the protocol has completed the RAC review process and was not selected for in-depth RAC review and public discussion, it may still raise issues that warrant careful consideration by the IBC and IRB. The RAC review process is not a substitute for the important institutional review of the protocol that must be carried out by the IBC and IRB.

As you proceed with the initiation of your protocol, the current reporting requirements set forth at Appendix M-I-C-1 of the *NIH Guidelines* require the Principal Investigator to submit additional documentation as specified to this office no later than 20 working days after enrollment of the first research participant. These requirements are as follows:

- a copy of the informed consent document approved by the Institutional Review Board (IRB);
- a copy of the protocol approved by the Institutional Biosafety Committee (IBC) and IRB;
- a copy of the final IBC approval from the clinical trial site;
- a copy of the final IRB approval;
- any modifications to the protocol as required by FDA;
- applicable NIH grant number(s);
- the FDA Investigational New Drug Application (IND) number; and
- the date of the initiation of the trial.

A copy of this and other sections of the *NIH Guidelines* that outline reporting requirements are enclosed.

The Internet site <http://www4.od.nih.gov/oba/> of the NIH OBA includes a copy of the complete *NIH Guidelines*, minutes of RAC meetings, and information about gene transfer research protocols registered with our office. Contact information for our office is as follows:

Office of Biotechnology Activities (OBA)
National Institutes of Health
6705 Rockledge Drive, Suite 750, MSC
Bethesda, Maryland 20892-7985
(All non-USPS mail should use zip code 20817)
Phone: 301-496-9838; Fax: 301-496-9839

Please let us know if you have any questions about the review of your submission or the requirements of the *NIH Guidelines*.

Sincerely,

Thomas Y. Shih, M.D., Ph.D.
Biotechnology Program Advisor

Attachments
Cc: RAC Chair and Members
Amy P. Patterson, M.D., Director, OBA
Stephen M. Rose, Ph.D., Deputy Director, Recombinant DNA Program, OBA
Stephanie L. Simek, Ph.D., Food and Drug Administration
John T. Balog, RBP, IBC Contact, Johns Hopkins University
Hayden Braine, M.D., IRB Chair, Johns Hopkins University

OUTCOME OF THE INITIAL REVIEW BY RAC MEMBERS

Human Gene Transfer Protocol: #0304-578

FDA IND:

Principal Investigator(s): Leisha Emens, M.D., Ph.D., Johns Hopkins University, Baltimore, MD 21231

Submitter: Same

Title: *A phase I vaccine safety and chemotherapy dose-finding trial of allogeneic GM-CSF-secreting breast cancer vaccine given in a specifically timed sequence with immunomodulatory doses of cyclophosphamide and doxorubicin*

In-depth RAC Review and Public Discussion Not Required: DeLuca, Friedmann, Bohn, P. Johnson, Lo, Kwan, Gooding, L. Johnson, Gelehrter, Simari, Wara, Childress

In-depth RAC Review and Public Discussion Required: None

Abstained: None

Recused: Sidransky, Linial

Are there comments by RAC members? No

Reporting Requirements
of the
NIH Guidelines for Research Involving Recombinant DNA Molecules
(Appendix M-I-C)

Appendix M-I-C-1. Initiation of the Clinical Investigation

No later than 20 working days after enrollment (see definition of enrollment in Section I-E-7) of the first research participant in a human gene transfer experiment, the Principal Investigator(s) shall submit the following documentation to NIH OBA: (1) a copy of the informed consent document approved by the Institutional Review Board (IRB); (2) a copy of the protocol approved by the Institutional Biosafety Committee (IBC) and IRB; (3) a copy of the final IBC approval from the clinical trial site; (4) a copy of the final IRB approval; (5) a brief written report that includes the following information: (a) how the investigator(s) responded to each of the RAC's recommendations on the protocol (if applicable); and (b) any modifications to the protocol as required by FDA; (6) applicable NIH grant number(s); (7) the FDA Investigational New Drug Application (IND) number; and (8) the date of the initiation of the trial. The purpose of requesting the FDA IND number is for facilitating interagency collaboration in the Federal oversight of human gene transfer research.

Appendix M-I-C-2. Additional Clinical Trial Sites

No research participant shall be enrolled (see definition of enrollment in Section I-E-7) at a clinical trial site until the following documentation has been submitted to NIH OBA: (1) Institutional Biosafety Committee approval (from the clinical trial site); (2) Institutional Review Board approval; (3) Institutional Review Board-approved informed consent document; (4) curriculum vitae of the principal investigator(s) (no more than two pages in biographical sketch format); and (5) NIH grant number(s) if applicable.

Appendix M-I-C-3. Annual Reports

Within 60 days after the one-year anniversary of the date on which the investigational new drug (IND) application went into effect, and after each subsequent anniversary until the trial is completed, the Principal Investigator (or delegate) shall submit the information set forth in (a), (b), and (c). When multiple studies are conducted under the single IND, the Principal Investigator (or delegate) may choose to submit a single annual report covering all studies, provided that each study is identified by its OBA protocol number.

(a) Clinical Trial Information. A brief summary of the status of each trial in progress and each trial completed during the previous year. The summary is required to include the following information for each trial: (1) the title and purpose of the trial; (2) clinical site; (3) the Principal Investigator; (4) clinical protocol identifiers, including the NIH OBA protocol number, NIH grant number(s) (if applicable), and the FDA IND application number; (5) participant population (such as disease indication and general age group, e.g., adult or pediatric); (6) the total number of participants planned for inclusion in the trial; the number entered into the trial to date; the number whose participation in the trial was completed; and the number who dropped out of the trial with a brief description of the reasons; (7) the status of the trial, e.g., open to accrual of subjects, closed but data collection ongoing, or fully completed, and (8) if the trial has been completed, a brief description of any study results.

(b) Progress Report and Data Analysis. Information obtained during the previous year's clinical and non-clinical investigations, including: (1) a narrative or tabular summary showing the most frequent and most serious adverse experiences by body system; (2) a summary of all serious adverse events submitted during the past year; (3) a summary of serious adverse events that were expected or considered to have causes not associated with the use of the gene transfer product such as disease progression or concurrent medications; (4) if any deaths have occurred, the number of participants who died during participation in the investigation and causes of death; and (5) a brief description of any information obtained that is pertinent to an understanding of the gene transfer product's actions, including, for example, information about dose-response, information from controlled trials, and information about bioavailability.

(c) A copy of the updated clinical protocol including a technical and non-technical abstract.

Appendix M-I-C-4. Safety Reporting

Principal Investigators must submit, in accordance with this section, Appendix M-I-C-4-a and Appendix M-I-C-4-b, a written report on: (1) any serious adverse event that is both unexpected and associated with the use of the gene transfer product (i.e., there is reasonable possibility that the event may have been caused by the use of the product; investigators should not await definitive proof of association before reporting such events); and (2) any finding from tests in laboratory animals that suggests a significant risk for human research participants including reports of mutagenicity, teratogenicity, or carcinogenicity. The report must be clearly labeled as a "Safety Report" and must be submitted to the NIH Office of Biotechnology Activities (NIH OBA) and to the local Institutional Biosafety Committee within the timeframes set forth in Appendix M-I-C-4-b.

Principal Investigators should adhere to any other serious adverse event reporting requirements in accordance with federal regulations, state laws, and local institutional policies and procedures, as applicable.

Principal Investigators may delegate to another party, such as a corporate sponsor, the reporting functions set forth in Appendix

M, with written notification to the NIH OBA of the delegation and of the name(s), address, telephone and fax numbers of the contact(s). The Principal Investigator is responsible for ensuring that the reporting requirements are fulfilled and will be held accountable for any reporting lapses.

The three alternative mechanisms for reporting serious adverse events to the NIH OBA are: by e-mail to oba@od.nih.gov; by fax to 301-496-9839; or by mail to the Office of Biotechnology Activities, National Institutes of Health, MSC 7985, 6705 Rockledge Drive, Suite 750, Bethesda, Maryland 20892-7985.

Appendix M-I-C-4-a. Safety Reporting: Content and Format

The serious adverse event report must include, but need not be limited to: (1) the date of the event; (2) designation of the report as an initial report or a follow-up report, identification of all safety reports previously filed for the clinical protocol concerning a similar adverse event, and an analysis of the significance of the adverse event in light of previous similar reports; (3) clinical site; (4) the Principal Investigator; (5) NIH Protocol number; (6) FDA's Investigational New Drug (IND) Application number; (7) vector type , e.g., adenovirus; (8) vector subtype, e.g., type 5, relevant deletions; (9) gene delivery method, e.g., *in vivo, ex vivo* transduction; (10) route of administration, e.g., intratumoral, intravenous; (11) dosing schedule; (12) a complete description of the event; (13) relevant clinical observations; (14) relevant clinical history; (15) relevant tests that were or are planned to be conducted; (16) date of any treatment of the event; and (17) the suspected cause of the event. These items may be reported by using the recommended Adverse Event Reporting Template available on NIH OBA's web site at: http://www4.od.nih.gov/oba/rac/documents1.htm, the FDA MedWatch forms, or other means provided that all of the above elements are specifically included.

Reports from laboratory animal studies as delineated in Appendix M-I-C-4 must be submitted in a narrative format.

Appendix M-I-C-4-b. Safety Reporting: Time frames for Expedited Reports

Any serious adverse event that is fatal or life-threatening, that is unexpected, and associated with the use of the gene transfer product must be reported to the NIH OBA as soon as possible, but not later than 7 calendar days after the sponsor's initial receipt of the information (i.e., at the same time the event must be reported to the FDA).

Serious adverse events that are unexpected and associated with the use of the gene transfer product, but are not fatal or life-threatening, must be reported to the NIH OBA as soon as possible, but not later than 15 calendar days after the sponsor's initial receipt of the information (i.e., at the same time the event must be reported to the FDA).

Changes in this schedule are permitted only where, under the FDA IND regulations [21 CFR 312(c)(3)], changes in this reporting schedule have been approved by the FDA and are reflected in the protocol.

If, after further evaluation, an adverse event initially considered not to be associated with the use of the gene transfer product is subsequently determined to be associated, then the event must be reported to the NIH OBA within 15 days of the determination.

Relevant additional clinical and laboratory data may become available following the initial serious adverse event report. Any follow-up information relevant to a serious adverse event must be reported within 15 calendar days of the sponsor's receipt of the information. If a serious adverse event occurs after the end of a clinical trial and is determined to be associated with the use of the gene transfer product, that event shall be reported to the NIH OBA within 15 calendar days of the determination.

Any finding from tests in laboratory animals that suggests a significant risk for human research participants including reports of mutagenicity, teratogenicity, or carcinogenicity must be reported as soon as possible, but not later than 15 calendar days after the sponsor's initial receipt of the information (i.e., at the same time the event must be reported to the FDA).

Appendix M-I-C-5. Confidentiality

Data submitted in accordance with Appendix M-I-C that are claimed to be confidential commercial or trade secret information must be clearly labeled as such. Prior to making its determination about the confidentiality of data labeled confidential commercial or trade secret, the NIH will contact the Principal Investigator or delegate to ascertain the basis for the claim and subsequently will notify the Principal Investigator or delegate of its final determination regarding the claim.

If NIH determines that the data so labeled are confidential commercial or trade secret and that their public disclosure would promote an understanding of key scientific or safety issues, the NIH will seek agreement from the appropriate party to release such data. Public discussion of scientific and safety issues raised by data submitted in accordance with Appendix M-I-C is vital to informing both investigators and human subjects about the safety of gene transfer research.

To protect the privacy of participants in gene transfer research, any serious adverse event or annual reports submitted to NIH OBA must not contain any information that would identify the human research participants.

From: "Rosenthal, Eugene (NIH/OD)" <rosenthg@od.nih.gov>
To: "'emensle@jhmi.edu'" <emensle@jhmi.edu>
Date: 5/13/03 1:48PM
Subject: Letter - Exemption of 0304-578

Dear Dr. Emens,

Inserted below is a copy of the letter that Dr. Tom Shih tried to send to you last night regarding the initial review of your submission by the RAC. A signed copy was sent to you today via standard mail. A copy of the signed letter was also sent to your IBC and IRB.

Please feel free to contact us if you have any questions.

Sincerely,
Eugene Rosenthal, Ph.D.
Biotechnology Program Advisor
Office of Biotechnology Activities
National Institutes of Health
6705 Rockledge Drive, Suite 750
Bethesda, Maryland 20892
(20892 for USPS only;
please use 20817 for other
delivery methods)
301-496-9838
301-496-9839 (fax)
rosenthg@od.nih.gov

-----Original Message-----
From: Shih, Tom (NIH/OD)
To: 'emensl@jhmi.edu '
Cc: Patterson, Amy (NIH/OD); Rose, Stephen (NIH/OD); Simek, Stephanie L (FDA); 'tfriedmann@ucsd.edu '; 'barkleye@hhmi.org '; 'm-bohn@northwestern.edu '; Brody, Baruch; 'jfc7c@cms.mail.virginia.edu '; 'ndeluca@pitt.edu '; 'demets@biostat.wisc.edu '; 'tdgum@umich.edu '; 'gooding@microbio.emory.edu '; 'Larry_Johnson@med.unc.edu '; 'JohnsonP@pediatrics.ohio-state.edu '; 'terry_kwan@brookline.mec.edu '; 'mlrac@fhcrc.org '; 'bernie@medicine.ucsf.edu '; 'powersm@gunet.georgetown.edu '; 'simari.robert@mayo.edu '; Sidransky, David; 'WaraD@peds.ucsf.edu '
Sent: 5/12/2003 10:39 PM
Subject: Letter - Exemption of 0304-578

May 12, 2003

Leisha A. Emens, M.D., Ph.D.
Assistant Professor of Oncology
The Sidney Kimmel Comprehensive Cancer Center
Johns Hopkins University
1650 Orleans St., Room 4M-90
Baltimore, MD, 21231

RE: Protocol #0304-578 entitled: A phase I vaccine safety and chemotherapy dose-finding trial of allogeneic GM-CSF-secreting breast cancer vaccine given in a specifically timed sequence with immunomodulatory doses of cyclophosphamide and doxorubicin

Dear Dr. Emens:

I am writing to notify you of the outcome of the initial review of your submission by members of the National Institutes of Health (NIH) Recombinant DNA Advisory Committee (RAC).

As you know, in accordance with Appendix M of the NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines), members of the RAC carried out an initial review of your submission to assess whether it raised any significant issues that warrant further review and discussion by the RAC in a public session. They were provided a copy of the entire protocol submission as well as a brief summary of the submission's main features. After review of this material and other relevant information, it was determined that your submission does not require an in-depth RAC review and public discussion.

As you know, during the initial review process, RAC members may request additional information or clarification about the submission. They also may have specific comments or suggestions about the protocol design, informed consent document, or other matters. These questions and comments were conveyed to you and you were provided an opportunity to address them. Since these comments represent the considered perspectives of individual members and do not constitute a consensus of the RAC, you are encouraged, but not required to, consider them further. This correspondence becomes part of the public record of the protocol submission and is available upon request to the investigator(s), sponsor (if applicable), Institutional Biosafety Committee (IBC) and Institutional Review Board (IRB) as well as members of the public. Requests should be directed to the NIH Office of Biotechnology Activities (OBA) via email (oba@od.nih.gov <mailto:oba@od.nih.gov>) or via telephone (301-496-9838). The NIH OBA protocol number should be included in any request.

The Principal Investigator and the institution are responsible for ensuring that no research participants are enrolled in the protocol until IBC approval, IRB approval and all applicable regulatory authorizations have been obtained. Please be mindful that even though the protocol has completed the RAC review process and was not selected for in-depth RAC review and public discussion, it may still raise issues that warrant careful consideration by the IBC and IRB. The RAC review process is not a substitute for the important institutional review of the protocol that must be carried out by the IBC and IRB.
As you proceed with the initiation of your protocol, the current reporting requirements set forth at Appendix M-I-C-1 of the NIH Guidelines require the Principal Investigator to submit additional documentation as specified to this office no later than 20 working days after enrollment of the first research participant. These requirements are as follows:

* a copy of the informed consent document approved by the Institutional Review Board (IRB);
* a copy of the protocol approved by the Institutional Biosafety Committee (IBC) and IRB;
* a copy of the final IBC approval from the clinical trial site;
* a copy of the final IRB approval;

* any modifications to the protocol as required by FDA;
* applicable NIH grant number(s);
* the FDA Investigational New Drug Application (IND) number; and
* the date of the initiation of the trial.

A copy of this and other sections of the NIH Guidelines that outline reporting requirements are enclosed.

The Internet site <<http://www4.od.nih.gov/oba/>> of the NIH OBA includes a copy of the complete NIH Guidelines, minutes of RAC meetings, and information about gene transfer research protocols registered with our office. Contact information for our office is as follows:

Office of Biotechnology Activities (OBA)
National Institutes of Health
6705 Rockledge Drive, Suite 750, MSC
Bethesda, Maryland 20892-7985
(All non-USPS mail should use zip code 20817)
Phone: 301-496-9838; Fax: 301-496-9839

Please let us know if you have any questions about the review of your submission or the requirements of the NIH Guidelines.

Sincerely,

/s/

Thomas Y. Shih, M.D., Ph.D.
Biotechnology Program Advisor

Attachments
Cc: RAC Chair and Members
Amy P. Patterson, M.D., Director, OBA
Stephen M. Rose, Ph.D., Deputy Director, Recombinant DNA Program, OBA
Stephanie L. Simek, Ph.D., Food and Drug Administration
John T. Balog, RBP, IBC Contact, Johns Hopkins University
Hayden Braine, M.D., IRB Chair, Johns Hopkins University

OUTCOME OF THE INITIAL REVIEW BY RAC MEMBERS

Human Gene Transfer Protocol: #0304-578

FDA IND:

Principal Investigator(s): Leisha Emens, M.D., Ph.D., Johns Hopkins University, Baltimore, MD 21231

Submitter: Same

Title: A phase I vaccine safety and chemotherapy dose-finding trial of allogeneic GM-CSF-secreting breast cancer vaccine given in a specifically timed sequence with immunomodulatory doses of

cyclophosphamide and doxorubicin

In-depth RAC Review and Public Discussion Not Required: DeLuca, Friedmann, Bohn, P. Johnson, Lo, Kwan, Gooding, L. Johnson, Gelehrter, Simari, Wara, Childress

In-depth RAC Review and Public Discussion Required: None

Abstained: None

Recused: Sidransky, Linial

Are there comments by RAC members? No

DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

Office of Biotechnology Activities
National Institutes of Health, MSC 7010
6705 Rockledge Drive, Suite 750
Bethesda, MD 20892-7010
301-496-9838 (Phone)
301-496-9839 (FAX)
http://www4.od.nih.gov/oba/

April 28, 2003

Leisha A. Emens, M.D., Ph.D.
Assistant Professor of Oncology
Johns Hopkins University School of Medicine
The Bunting Blaustein Cancer Research Building
1650 Orleans Street/Room 4M-90
Baltimore, MD 21231-1000

Re: Registration of Protocol # 0304-578 entitled: *A Phase I Vaccine Safety and Chemotherapy Dose-Finding Trial of an Allogeneic GM-CSF-secreting Breast Cancer Vaccine Given in a Specifically Timed Sequence with Immunomodulatory Doses of Cyclophosphamide and Doxorubicin*

Dear Dr. Emens:

This letter is to acknowledge receipt of documentation for the above referenced protocol by the Office of Biotechnology Activities (OBA).

Pursuant to the *NIH Guidelines for Research Involving Recombinant DNA Molecules*, OBA has sent a summary of the protocol to each member of the Recombinant DNA Advisory Committee (RAC) for a recommendation as to whether the protocol requires public discussion by the full RAC. OBA will notify you in writing whether the protocol does or does not require in-depth review and public discussion at a RAC meeting.

Please refer to the above-referenced protocol number (0304-578) in any correspondence with OBA. Please feel free to contact this office if you have any questions.

Sincerely,

Eugene Rosenthal, Ph.D.
Biotechnology Program Advisor

DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

National Institutes of Health
Bethesda, Maryland 20892

Office of Biotechnology Activities
National Institutes of Health, MSC 7985
6705 Rockledge Drive, Suite 750
Bethesda, MD 20892-7985
301-496-9838 (Phone)
301-496-9839 (FAX)
http://www4.od.nih.gov/oba

April 17, 2003

Leisha A. Emens, M.D., Ph.D.
1650 Orleans Street, Room 4M90
Bunting Blaustein Cancer Research Building
Baltimore, MD 21231-1000

Re: Submission of an IND entitled: *A Phase I Vaccine Safety and Chemotherapy Dose Finding Trial of an Allogeneic GM-CSF-secreting Breast Cancer Vaccine Given in a Specifically Timed Sequence with Immunomodulatory Doses of Cyclophosphamide and Doxorubicin*

Dear Dr. Emens:

According to an agreement between the National Institutes of Health (NIH) Office of Biotechnology Activities (OBA) and the Center for Biologics Evaluation and Research of the Food and Drug Administration, we have been notified of your April 11, 2003 submission for an investigational new drug application (IND). This IND appears to employ recombinant DNA technology to modify human cells and, therefore, must be submitted to OBA. According to the *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)* (Appendix M-I-A), the following information should be submitted to our office:

(1) A cover letter on institutional letterhead, signed by the Principal Investigator(s), that: (i) acknowledges that the documentation submitted to NIH OBA complies with the requirements set forth in Appendix M-I-A, *Requirements for Protocol Submission*; (ii) identifies the Institutional Biosafety Committee (IBC) and Institutional Review Board (IRB) at the proposed clinical trial site(s) responsible for local review and approval of the protocol; and (iii) acknowledges that no research participant will be enrolled (see definition of enrollment in Section I-E-7 of the *NIH Guidelines*) until the RAC review process has been completed (see Appendix M-I-B, *RAC Review Requirements*); IBC approval (from the clinical trial site) has been obtained; IRB approval has been obtained; and all applicable regulatory authorizations have been obtained; (2) The scientific abstract; (3) The non-technical abstract; (4) The proposed clinical protocol, including tables, figures, and relevant manuscripts; (5) Responses to Appendices M-II through M-V, *Description of the Proposal, Informed Consent, Privacy and Confidentiality, and Special Issues*. Responses to Appendices M-II through M-V may be provided either as an appendix to the clinical protocol or incorporated in the clinical protocol. If responses to Appendices M-II through M-V are incorporated in the clinical protocol, each response must refer to the appropriate Appendix M-II through M-V; (6) The proposed informed consent document (see Appendix M-III, *Informed Consent*); (7) Curriculum vitae of the principal investigator(s) (no more than two pages in biographical sketch format).

Please note that the latest version of the *NIH Guidelines*, containing more detailed submission information, can be found at OBA's Internet site (http://www4.od.nih.gov/oba/rac/guidelines/guidelines.html).

Thank you for your attention to this matter. If you have any questions, please contact me via email (rosenthg@od.nih.gov) or at 301-496-9838.

Sincerely,

Eugene Rosenthal, Ph.D.
Biotechnology Program Advisor

cc: Amy P. Patterson, M.D.; Director, OBA
Stephen M. Rose, Ph.D.; Deputy Director, Recombinant DNA Program, OBA
Stephanie Simek, Ph.D.; Food and Drug Administration (via e-mail)

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Commentary

A New Twist on Autologous Cancer Vaccines

Leisha A. Emens

Leisha A. Emens, M.D., Ph.D.; Assistant Professor of Oncology; The Sidney Kimmel Comprehensive Cancer Center; Bunting-Blaustein Cancer Research Building; 1650 Orleans Street, Room 4M90; Baltimore, Maryland 21231-1000 USA; Tel.: 410.502.7051; Fax: 410.614.8216; Email: emensle@jhmi.edu

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Physicians have had a long-standing interest in marshalling the cancer patient's own immune system to effect tumor rejection. The use of cancer vaccines to activate an endogenous antitumor immune response has the advantages of exquisite tumor specificity, low toxicity, and potential durability due to the phenomenon of immunologic memory. Moreover, cancer vaccines are an attractive complement to the standard cancer treatment modalities of surgery, radiation therapy, and chemotherapy, offering a non-toxic treatment strategy that is likely to be non-cross resistant. Even with these advantages, the use of therapeutic cancer vaccines also poses significant challenges. Their efficacy is hampered by the extent of the tumor burden, relatively well entrenched mechanisms of tumor-specific immune tolerance, and the potential plasticity of the tumor cells themselves. From a practical point of view, the development of tumor vaccines is further limited by the technical limitations posed by the nature of the vaccination platform itself. In this issue of *Cancer Biology & Therapy*, Lasalvia-Prisco and colleagues report the results of the first clinical trial testing a novel vaccine formulation utilizing an autologous hemoderivative for the treatment of advanced solid malignancies.[1] While clearly preliminary, their approach is intriguing because it circumvents many of the practical obstacles to the development of effective vaccines for cancer therapy.

Tumor vaccine formulations can be broadly divided into those that are well defined, and those that are.[2] Well-defined tumor vaccines contain known tumor antigens; examples include peptide-based, protein-based, and plasmid DNA vaccines. These vaccines offer the advantages of relative ease of manufacture, clear targets for the monitoring of immune responses to vaccination, and a good safety record to date. One disadvantage to the use of precisely targeted tumor vaccines is that many tumor antigens seem to activate antigen-specific immunity that is incapable of mediating a tumor rejection response. This concept is supported by the results of studies characterizing the natural and vaccine-induced immune responses in melanoma patients who continue to have disease progression despite the presence of antigen-specific tumor immunity.[2-7] A second disadvantage to highly targeted cancer immunotherapies is that they favor the selection of antigen loss variants, ultimately resulting in the outgrowth of a subpopulation of antigen-negative tumor cells that are by definition resistant to therapy.[8-10]

Tumor vaccines that are less well defined are generally formulated either directly from tumor cells themselves to make a cellular vaccine, or are derived from tumor cells as a crude preparation of viral lysate or heat shock protein (HSP)-peptide complexes.[2] There are two primary advantages to cancer vaccine formulations that are less well defined. First, these vaccine platforms deliver a variety of tumor antigens. By definition, they are capable of directing the immune response simultaneously to multiple antigens, greatly decreasing the probability of immune-mediated selection of antigen loss variants. Second, the menu of antigens delivered can include both known and as yet unknown tumor antigens. The sheer number of antigens delivered thus increases the likelihood of activating the immune system to recognize an antigen that can mediate tumor rejection. One disadvantage to the use of undefined antigen vaccines relates to the primary antigen source. The use of autolgous tumor cells is often preferred due to the possiblity that critical targets for immune-mediated tumor rejection are unique to each tumor. However, sufficient numbers of autologous tumor cells are frequently not available to support full vaccination regimens, leading some to investigate the use of allogeneic tumor cells to deliver tumor antigens common to a given histology. A second disadvantage of using relatively undefined vaccine platforms is that there is often no clear target for monitoring vaccine-induced immmune responses. Some investigators have used the development of delayed type hypersensitivity (DTH) to autologous tumor as an informative measure of vaccine-induced antitumor immunity.[11-22] Again, this is possible only when autologous tumor is available for processing, and this is frequently not the case for advanced solid tumors. A third drawback to less defined vaccine formulations is that the quality of the manufacturing process may be difficult to ensure.

In particular, the antigenic content of autologous tumor cell-based formulations will be unique. Appropriate measures of manufacturing consistency and potency may thus be more difficult to define for cell-based as opposed to highly targeted cancer vaccines.

Lasalvia-Prisco and colleagues describe a novel vaccine formulation derived from the arterial blood of advanced solid tumor patients. They develop a procedure for manufacturing and partially characterizing an autologous hemoderivative, and then test it as a cancer vaccine in a clinical trial involving patients with a variety of advanced solid tumors. The processing of the vaccine itself is simple. It is derived from 20 milliliters of femoral arterial blood. After sedimentation at 37°C, the supernatant of plasma and cells is subjected to hypotonic shock, followed by freezing. Twenty-four hours later the preparation is thawed, exposed to 100°C for 10 minutes, and filtered over cellulose acetate. A crude analysis of the vaccine preparation showed it to consist of a minimum of five protein fractions, with a major homogeneous protein component of approximately 50,000 kD. Although they were present prior to processing, heat shock proteins and known tumor markers were not detected in the final product.

Although it remains relatively uncharacterized, this vaccine platform in principle offers multiple advantages. First, the small quantity of arterial blood required is quickly and safely accessible by femoral arterial puncture. Second, the manufacturing process is relatively simple and cost-effective, requiring minimal manipulation and no direct chemical or genetic modification of the cellular component prior to processing. Finally, and most importantly, the patient himself is a renewable source of therapeutic material. At the time of tumor recurrence, an updated vaccine product that accurately reflects that antigenic profile of the tumor at that point in time could be easily obtained and prepared.

The investigators designed the clinical trial to overcome some other limitations to the efficacy of therapeutic cancer vaccines. They include GM-CSF, a cytokine that is well known for its immune-stimulating properties, as a local vaccine adjuvant. They also incorporate a low dose of intravenous Cyclophosphamide three days prior to vaccination. Although chemotherapeutic immunomodulation is not widely used in cancer vaccine trials, there is substantial preclinical and clinical evidence to suggest that Cyclophosphamide can augment the induction of antigen-specific immunity.[18,23-26] Importantly, the trial also included a control group that received Cyclophosphamide and GM-CSF but no autologous hemoderivative. The vaccinated group had a higher frequency of stable (SD) or responding (PR) disease than did the control group ($p < 0.001$). Also, the vaccinated patients demonstrated a correlation between clinical response (SD + PR) and the development of DTH to the autologous hemoderivative of at least 5 mm in diameter ($p < 0.02$). Moreover, histologic analysis of responding metastatic lesions were characterized by stromal fibrosis and CD^{3+} T cell infiltration not characteristic of pre-treatment biopsies.

The mechanism underlying the reported bioactivity of this treatment approach remains an open question. Although no HSPs are found in the final vaccine product, the overall vaccine preparation is reminiscent of HSP cancer vaccines.[27] It is possible that a stress-related protein present in the vaccine preparation delivers tumor-derived antigens present in the arterial blood in a form capable of activating T cell-dependent immunity. This is supported by the development of DTH to the hemoderivative, and by the presence of T cells infiltrating the responding tumors. It would be more strongly supported by additional measures of antitumor immunity, such as DTH to autologous tumor where available (rather than the autologous hemoderivative), or cellular immune responses to a defined antigen known to be present in the patients tumor (by ELISPOT).

Interestingly, the manufacturing process includes a step of hypo-osmotic shock. Hypo-osmotic shock is known to activate monocytes and macrophages, enhancing phagocytosis and the secretion of cytokines such as interleukin 1 and interleukin 6.[28,29] The contribution of this step to the bioactivity of the vaccine is unclear, but it could provide a vaccine adjuvant that is an integral component of the vaccine itself.

The most striking histopathologic feature of responding lesions is the presence of marked fibrosis. The extent of this fibrotic response suggests an additional mechanism distinct from that mediated by T cell against the tumor cells themselves. The observed histology strongly argues for a therapeutic effect that shifts the balance of interactions between the tumor cells and the supporting stroma to favor tumor regression. Carefully elucidating the regulatory pathways underlying this aspect of the hemoderivative's bioactivity should facilitate the development of informative surrogate measures of clinical response for use in future clinical trials.

In summary, Lasalvia-Prisco and colleagues have described a novel cancer vaccine platform consisting of an autologous hemoderivative, with a suggestion of clinical response. These results are preliminary, and require confirmation in larger trials and by other investigators. Further characterization of the critical parameters of vaccine formulation and the mechanism of bioactivity will facilitate the development of more informative clinical trials. If these results are confirmed and extended, this vaccine platform represents an exciting development in the field of cancer immunotherapy. The ability to re-derive a potent vaccine in response to the changing antigenic profile of an evolving metastatic tumor is a powerful and unique feature of this vaccine platform. Clearly, an active, individualized cancer vaccine that is cost-effective and simple to manufacture would be a welcome addition to the treatment armamentarium for metastatic solid tumors.

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Models of Anti-Cancer Therapy

Cancer Vaccines

An Old Idea Comes of Age

Leisha A. Emens[1,*]
Elizabeth M. Jaffee[1,2,3]

Departments of [1]Oncology, [2]Pathology, and [3]Immunology; The Johns Hopkins University School of Medicine and the Sidney Kimmel Comprehensive Cancer Center; 1650 Orleans Street; Baltimore, Maryland USA

*Correspondence to: Leisha A. Emens, M.D., Ph.D.; The Sidney Kimmel Comprehensive Cancer Center; Bunting-Blaustein Cancer Research Building; 1650 Orleans Street, Room 4M90; Baltimore, Maryland 21231-1000 USA; Tel.: 410.502.7051; Fax: 410.614.8216; Email: emensle@jhmi.edu

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KEY WORDS

Cancer vaccines, Clinical trials, Immune tolerance, Tumor antigens, Antigen identification

Elizabeth M. Jaffee, M.D.

ABBREVIATIONS

AICD	Activation induced cell death
APC	Antigen presenting cell
BMT	Bone marrow transplant
β-HCG	β-Human chorionic gonadotropin
CEA	Carcinoembryonic antigen
CML	Chronic myelogenous leukemia
CTL	Cytotoxic T lymphocyte
DFS	Disease-free survival
DTH	Delayed type hypersensitivity
GM-CSF	Granulocyte-macrophage colony-stimulating factor
HA	Hemagglutinin
IFN-γ	Interferon-γ
KLH	Keyhole limpet hemocyanin
MHC	Major histocompatibility complex
TIL	Tumor infiltrating lymphocytes
PBL	Peripheral blood lymphocytes
SAGE	Serial analysis of gene expression
SEREX	Serologic analysis of recombinant cDNA expression
TNFR	Tumor necrosis factor receptor

ABSTRACT

Cancer vaccines are at the forefront of novel, targeted approaches to cancer treatment. Low toxicity, the potential for circumventing drug cross-resistance, and the potential for persistence of the antitumor effect due to immunologic memory represent a mandate for accelerated clinical development. Advances in molecular immunology have suggested approaches for overcoming the formidable mechanisms of immune tolerance that are pre-established in cancer patients, and many have already been tested in preclinical models. Also, early studies revealed that not all tumor antigens are created equal, and identifying those capable of eliciting immune-mediated tumor rejection is essential to the development of effective recombinant cancer vaccines. While early trials have generally resulted in disappointing clinical outcomes, they have yielded insight into the critical parameters for the design of cancer vaccine trials and provided powerful reagents for tumor antigen identification. By utilizing the lessons learned from the research laboratory and these early clinical trials, informative second generation vaccine trials should have a high likelihood of success. Clinical protocols that consider how best to incorporate therapeutic cancer vaccines into the current standard of care should allow cancer vaccines to take their place alongside traditional cancer treatment modalities in oncology practice.

INTRODUCTION

The use of immunization to prevent acute infectious diseases is one of the success stories of modern medicine. It has essentially eradicated smallpox and polio, and has dramatically decreased the morbidity and mortality of other infectious diseases. Despite success in disease prevention, the use of vaccines to treat established, chronic disease (including chronic viral infections and cancer) has not been successful. There are several explanations for the inherent difficulties of immunotherapy compared to immunoprophylaxis. First, the pivotal antigens required for the induction of effective antiviral and antibacterial immunity are few and well characterized immunologically. In contrast, relatively little is known about the critical immune targets of vaccine-activated immune rejection of genetically complex, transformed host cells. Second, the humoral immune response typically eliminates acute infections, whereas T cell-mediated immune responses are thought to eradicate established cancer or chronically infected host cells. Third, the vaccine-mediated induction of immunity prior to antigen exposure occurs on a slate that is immunologically clean. In contrast, inducing a targeted immune response in chronic disease requires disrupting established immunoregulatory mechanisms that maintain a parasitic relationship between the affected cell and the host. Finally, the pre-existing tumor burden itself represents an additional hurdle that must be surmounted in the therapeutic setting.

MECHANISMS OF ANTITUMOR IMMUNITY

Interest in harnessing the immune system for cancer treatment dates back to 1893 when William Coley reported the regression of soft tissue sarcoma in patients with acute bacterial infection, then tested bacterial extracts to stimulate antitumor immune responses.[1] Subsequent efforts to develop cancer vaccines were stymied by the prevailing ignorance of basic immunology. The recent revolution in biotechnology created the tools to both elucidate the molecular mechanisms of immunoregulation and further define the principles of tumor immunology. In the presence of a pro-inflammatory or danger signal, activated professional antigen presenting cells (APC) initiate $CD4^+$ and $CD8^+$ T cell responses by capturing, endocytosing, and processing tumor antigens released by cancer cells (Fig. 1). Antigens are processed by the endosome into MHC Class II-binding peptides of 12 to 20 amino acids, and by the proteosome into MHC Class I-binding peptides of

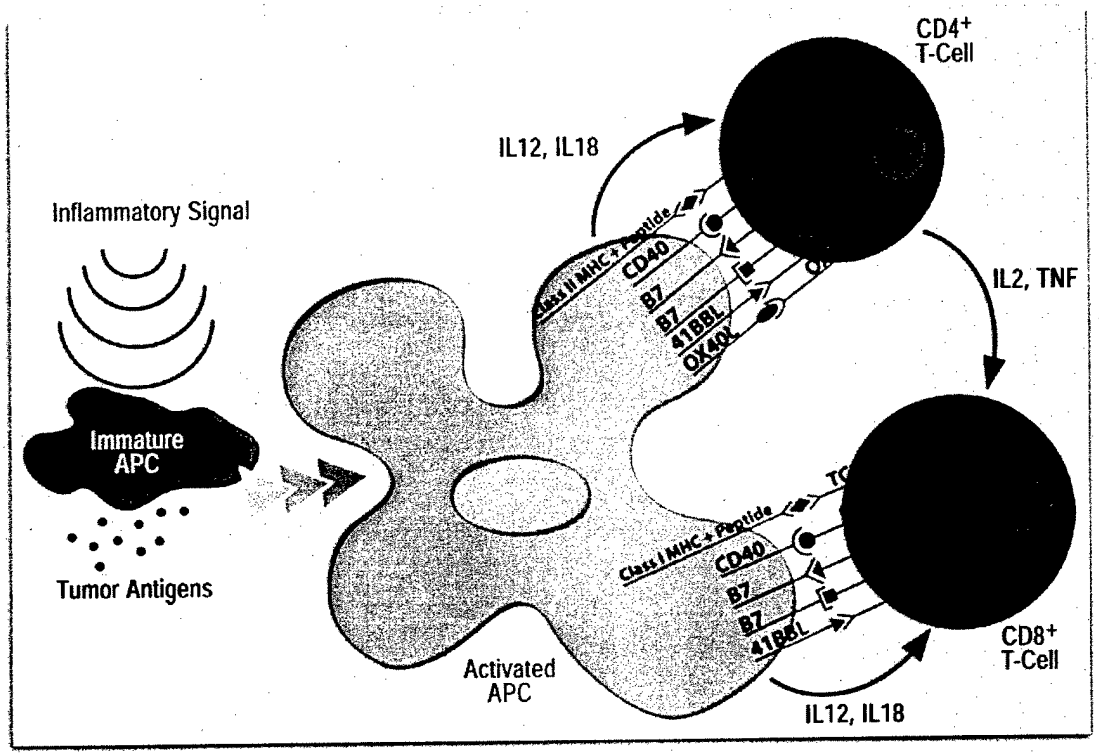

Figure 1. Activation of Antitumor Immunity. Professional antigen-presenting cells (APC) become activated in the presence of a proinflammatory signal. They then capture, endocytose, and process tumor antigens delivered by a vaccine or released by tumor cells. Concomitantly, professional APC upregulate a variety of costimulatory molecules (including B7-1, B7-2, CD40, CD27 ligand, OX40 ligand, LIGHT, and 41BB ligand) that can provide a second activation signal upon binding to cognate receptors on T cells. Tumor antigens are simultaneously presented to CD4+ and CD8+ T cells in the context of MHC Class II and MHC Class I, thus activating the antigen-specific immune response.

8 to 10 amino acids.[2] TAP transporters transfer the MHC Class I peptide epitopes to the endoplasmic reticulum, where they associate with MHC Class I molecules and are translocated to the cell surface. Professional APC simultaneously present tumor antigens to both CD4+ and CD8+ T cells in the context of MHC Class II and MHC Class I respectively, thus cross-priming the antigen-specific immune response.[3] Activated CD4+ T cells initiate and amplify the CD8+ T cell response directly by providing stimulatory cytokines, and indirectly by upregulating a variety of costimulatory molecules on the APC that provide a second signal for T cell activation. Activated CD8+ T cells then acquire the potential to lyse tumor cells.[4] Notably, in the absence of a danger or inflammatory signal, critical costimulatory molecules are not upregulated on the surface of the APC, resulting in downregulation of the T cell response.[5] The influence of the inflammatory milieau at the time of immune priming and activation thus has clear implications for the clinical development of vaccine-based approaches to cancer treatment.

TUMOR ANTIGENS AND VACCINATION PLATFORMS

The success of immunization in infectious disease suggests that identification of pivotal antigens for immune-mediated tumor rejection will facilitate the development of highly targeted and effective vaccines for cancer therapy and prevention. The strength of a candidate antigenic target for cancer immunotherapy is determined by several characteristics: its tissue expression profile, the diversity, scope, and avidity of its T cell repertoire, the presence or absence of pre-existing immune tolerance, and the commonality of the antigen between patients and distinct tumor types.[6] The focus of tumor antigen identification has historically been on T cell targets. However, in light of increasing evidence that B cell-mediated immunity may participate in tumor rejection,[7,8] the focus is increasingly on the identification of tumor antigens that elicit both B cell and T cell responses. The different classes of tumor antigens are summarized in Table 1.

Vaccine formulations range from the highly targeted, such as peptide-based immunization, to the less well defined, including whole tumor cells and tumor cell lysates (Table 2). In general, current approaches to cancer vaccine design are based on directly manipulating B cells, T cells, or professional APC.[9] Approaches for activating humoral immunity have included vaccinating with tumor-specific carbohydrate antigens delivered by whole tumor cells or as conjugates with keyhole limpet hemocyanin (KLH). T cells can be activated directly by the vaccine, either by genetically modifying tumor cells to express costimulatory molecules or by genetically modifying professional APC with tumor antigens. Alternatively, T cells can be activated by immunizing with professional APC after direct loading of empty MHC with relevant tumor antigen either in vitro or in vivo. T cells can also be activated indirectly by the sustained local delivery of cytokines to recruit professional APC to the site of antigen deposition in vivo. The early systematic analysis in preclinical models identified granulocyte-macrophage colony-stimulating factor (GM-CSF) as the most potent cytokine in this regard.[10] GM-CSF-secreting tumor vaccines have been tested in Phase I clinical trials in melanoma, renal cell carcinoma, prostate cancer, and pancreatic cancer.[11-14] These trials have demonstrated the safety and bioactivity of this vaccine approach, and have suggested the potential for clinical benefit.

Table 1 **TUMOR ANTIGENS**

Type of Antigen	Tumor Type
Cancer Testis Antigens	
MAGE	Melanoma, Breast carcinoma, Esophageal carcinoma, Gastric carcinoma, GBM, HCC, Head/Neck carcinoma, Rhabdomyosarcoma
BAGE	Melanoma, Breast carcinoma, Esophageal carcinoma, Gastric carcinoma, HCC, Rhabdomyosarcoma
GAGE	Melanoma, Esophageal carcinoma, Gastric carcinoma, GBM, Prostate carcinoma, HCC, Rhabdomyosarcoma
RAGE	Renal carcinoma, sarcoma
NY-ESO-1	Melanoma, Prostate carcinoma, Breast carcinoma, TCC, NSCLC Tissue-specific Differentiation Antigens
Tissue-specific Differentiation Antigens	
Tyrosinase	Melanoma
MART-1/melan-A	Melanoma
gp100	Melanoma
TRP-1, TRP-2	Melanoma
PSA	Prostate carcinoma
Mutated Gene Products	
CDK-4-R24C	Melanoma
β-catenin	Colon carcinoma
p53	NSCLC, Colon carcinoma, Breast carcinoma
k-*ras*	Pancreatic carcinoma, Colon carcinoma, NSCLC, Esophageal carcinoma
MUM-1	Multiple myeloma, lymphoma
bcr-abl	CML, AML
Overexpressed Self Antigens	
HER-2/*neu*	Breast carcinoma
Proteinase-3	CML, AML
Mucin-1	Multiple myeloma
WT-1	CML, AML, ALL
MART-1/Melan-A	Melanoma
Viral Antigens	
HPV	Cervical carcinoma, head and neck carcinoma
HBV, HCV	Hepatocellular carcinoma
EBV	Burkitt's lymphoma, nasopharyngeal carcinoma, PTLD
Idiotypes	
Ig idiotype	B cell lymphoma
TCR idiotype	T cell lymphoma

Abbreviations: PSA, prostate-specific antigen; HPV, human papilloma virus; HBV, hepatitis B virus; HCV, hepatitis C virus; EBV, Epstein Barr virus; Ig, immunoglobulin; TCR, T cell receptor; GBM, glioblastoma multiforme; HCC, hepatocellular carcinoma; TCC, transitional cell carcinoma; NSCLC, non-small cell lung carcinoma; CML, chronic myelogenous leukemia; AML, acute myelogenous leukemia; ALL, acute lymphocytic leukemia; PTLD, post-transplant lymphoproliferative disease.

BARRIERS TO SUCCESSFUL IMMUNOTHERAPY FOR CANCER

A number of early trials have hinted at the promise of cancer vaccines (Table 3).[15-22] They have also illustrated the importance of considering tumor burden, immune tolerance, vaccine formulation, and surrogate markers of response in clinical trial design. Zinkernagel and Old elegantly described the influence of tumor burden on the development of effective antitumor immunity. Very small tumors are often ignored by the immune system, growing in the periphery without accessing the lymphatic tissues. They thus neither activate nor tolerize tumor-specific T cells, a phenomenon referred to as "sneaking through".[23] Once the tumor has reached sufficient size, it infiltrates the local lymphoid tissue, thus activating a late antitumor immune response. Tumor rejection is then determined by the relative growth kinetics and physical burden of tumor cells compared to the intensity and diversity of the effector T cell response induced.[24] Superimposed on this imbalance are the mechanisms by which tumors evade the immune system. These include the elaboration of inhibitory cytokines (interleukin-10, transforming growth factor-β and prostaglandin E_2), and the expression of molecules such as CD95L that can induce the apoptosis of tumor infiltrating lymphocytes (TIL).[25] Tumors can also down-regulate the expression of tumor-specific antigens targeted by a vaccine or therapeutic antibody, resulting in the outgrowth of antigen loss variants.[26,27] Furthermore, tumors can downregulate various components of the antigen processing machinery (including MHC Class I molecules themselves, various proteosome subunits, and the TAP transporter),[25] a phenomenon that has been correlated with poor clinical outcome.[28]

Immune tolerance represents the second major barrier to the effectiveness of cancer vaccines (reviewed in ref. 29). Unlike infectious challenges, tumor cells arise endogenously. Thus, with the exception of de novo genetic mutations reflected in the transcriptional profile, the majority of tumor antigens are recognized as self. As a consequence, critical elements of the tumor-specific T cell repertoire are often either deleted centrally in the thymus (in the case of tumor antigens recognized as self) or eliminated peripherally by deletion or activation-induced cell death (AICD) (in the case of widely disseminated systemic tumor).[29] Alternatively, tumor cells can render tumor-specific T cells unresponsive or anergic by presenting antigen in the absence of costimulatory signals.[29] Tumor antigens can also be ignored by the immune system, either by virtue of very low expres-

sion levels or compartmentalization away from lymphatic tissues (i.e., expression limited to embryologic development).[29] If T cells do become activated, a phenotypically skewed cytokine/chemokine receptor profile can render them ineffective by virtue of cytokine deviation and ineffective trafficking.[29] Finally, both tolerizing dendritic cells and $CD4^+CD25^+$ regulatory T cells can negatively impinge on antitumor immune responses.[29] A thorough understanding of these diverse mechanisms of immune tolerance and their impact on the T cell repertoire available for immune manipulation will facilitate the development of innovative, targeted approaches for overcoming them.

Third, despite the vast array of tumor antigens that has been identified, only a very few are candidate tumor rejection antigens. A tumor rejection (or regression) antigen is an antigen preferentially associated with a cancer that can be effectively targeted by the immune system to destroy tumor cells, resulting in clinically relevant immune-mediated antitumor responses.[6] That not all tumor antigens are also targets for rejection is well illustrated by the lack of concordance between the induction of antigen-specific immunity and tumor regression observed in multiple clinical cancer vaccine trials.[30-34] This concept is best illustrated by the natural immune response of some melanoma patients who, despite the presence of significant numbers of functional cytotoxic effector T cells specific for the melanocyte-specific antigen MART-1/Melan-A, have progressive disease.[35,36] The numbers of MART-1/Melan-A T cells in these patients can be augmented with targeted vaccines, but this does not influence the immunodynamics of the antitumor response in a clinically significant way.[30,32,35-37] A similar phenomenon is reported for gp100-specific peptide vaccination.[38] Thus, a key task is to identify pivotal tumor rejection targets, and then to focus clinical vaccine development on those critical tumor antigens.

Table 2 **SUMMARY OF DIFFERENT VACCINE FORMULATIONS**

Formulation	Immunogenicity	Potential for Toxicity	Requirement for HLA Match
Peptide + Adjuvant	low	low	yes
Plasmid DNA	low	low	no
Recombinant Virus Replication-deficient pox virus Adenovirus Adeno-associated virus Herpesvirus Retrovirus/Lentivirus	high	high	no
Recombinant Bacteria Listeria monocytogenes Salmonella typhimurium Shigella Mycobacterium bovis BCG	high	high	no
Dendritic Cells In vitro peptide loaded In vitro tumor lysate loaded Dendritic cell-tumor cell fusion	high	low	yes
Tumor Cells Autologous Allogeneic Bystander	moderate to high	low	no
Heat Shock Proteins	high	low	no

In addition to the challenges posed by the intricate immunobiology of the host-tumor interaction, the immaturity of the field of cancer immunotherapy places significant constraints on several key aspects of clinical trial design. The first limitation to clinical trial design is the lack of reliable immunologic surrogate markers of clinical response. The most commonly accepted test of vaccine-induced antitumor immunity is the delayed type hypersensitivity (DTH) test against autologous tumor, which correlates with survival in several trials.[15-18] While the undisputed strength of DTH analysis is the use of patient-derived autologous tumor to assess immunity in vivo, the limited availability of autologous tumor often prevents its use. As an alternative, the combination of ELISPOT and MHC tetramer analysis is now under development for the serial assessment of the dynamics of the antigen-specific $CD8^+$ T cell response, yielding information about both function and numbers of antigen-specific T cells.[39] While the advantage is that peripheral blood lymphocytes (PBL) are relatively easy to obtain, peripheral T cell populations might not reflect what is happening at the tumor site. Finally, a correlation between the development of antibody titers and clinical benefit has been demonstrated in at least three trials. In one study, prolonged survival was associated with the induction of GM2 IgM antibody responses in Stage III melanoma patients immunized with the ganglioside vaccine GM2/BCG after low dose Cyclophosphamide ($p = 0.02$).[40] A second study demonstrated a survival benefit for Stage II melanoma patients immunized with an allogeneic cellular melanoma vaccine who developed IgM antibody titers specific for TA90 greater than 1:800 ($p = 0.0013$).[41] The third study demonstrated a correlation between improved survival and the development of significant β-human chorionic gonadotropin (β-HCG) antibody titers in metastatic colon cancer patients immunized with a β-HCG peptide vaccine conjugated to diphtheria toxin (DT) ($p = 0.0002$).[42]

The second limitation to clinical trial design is the lack of informative predictors of immunologic or clinical response. The simplest example of predictors of immunologic response is the use of HLA genotype to identify patients that will respond to HLA-specific peptide vaccines, the most common being HLA-A2. The feasibility of identifying predictors of clinical response for other cancer vaccine platforms is suggested by two recent reports. First, the Southwest Oncology Group tested an allogeneic melanoma vaccine compared to observation alone in patients with intermediate thickness, lymph-node negative melanoma after surgical resection. A prospective analysis revealed a statistically significant clinical benefit in vaccinated HLA-A2 and HLA-C3 positive patients compared to HLA-A2 and HLA-C3 negative vaccinees, with 5-year disease-free survival (DFS) rates of 77% and 64% respectively ($p = 0.004$).[22] Second, Wang and colleagues recently demonstrated the feasibility of defining tumor profiles as predictors of clinical response.[43] They classified metastatic melanomas by cDNA profiling, and identified two subsets of melanomas. Although neither of these two profiles correlated with clinical response, about 30 genes predictive of clinical response to immunotherapy were identified. Interestingly, approximately half are related to T cell regulation. This is clearly an area for further research.

Table 3 **SUMMARY OF RECENT PHASE II AND PHASE III CANCER VACCINE TRIALS**

Vaccine	Patient Population	Phase	N	Intervention	Results
Hapten-modified autologous in melanoma tumor cell vaccine (ref. 15)	Stage III/IV melanoma after regional lymphadenectomy	II	77	Vaccine	Improved 5 year survival in vaccinated patients > 50years, (p = 0.011), and who developed DTH > 5mm (p = 0.031)
Allogeneic melanoma tumor cell vaccine (ref. 16)	Metastatic melanoma after complete resection	II	77	Vaccine + BCG	Improved sruvival with the development of DTH and αTA90 IgM (p < 0.0001 for OS)
Sialyl-Tn-KLH + DETOX-B adjuvant (ref. 17)	Metastatic breast cancer	II	23	Cy + Vaccine vs. Vaccine	Increased sialyl-TN immune responses with Cy pretreatment
Autologous colon cancer tumor vaccine + BCG (ECOG 5283) (ref. 18)	Stage II/III colon carcinoma after resection	III	412	Vaccine vs. Observation	No arm differences (p = 0.73 for OS) improved survival if vaccine site reaction > 1 cm (p = 0.003 for OS)
Allogeneic melanoma tumor cell vaccine (ref. 19)	Metastatic melanoma after complete resection relapsed on vaccine therapy (trial in ref. 16)	III	194	Vaccine vs. Vaccine + BCG	Improved survival in relapsed patients reinduced with more frequent vaccinations and more BCG (p = 0.0178)
Conjugated ganglioside (GM2—KLH) + QS-21 adjuvant (ref. 20)	Stage IIB/III melanoma after resection	III	880	Vaccine vs. High dose IFNα2B	IFNα2B superior to vaccine (p = 0.009 for OS)
Allogeneic melanoma tumor cell vaccine (ref 21,22)	Stage IB/IIA melanoma after resection	III	689	Vaccine vs. Observation	No arm differences; Improved survival in HLA-A2 and HLA-A3 vaccinees (p = 0.004)

Abbreviations: DTH, delayed type hypersensitivity; BCG, Bacille Calmette-Guerin; KLH, keyhole limpet hemocyanin; Cy, Cyclophasphamide; IFNα2B, interferon-α; OS, overall survival; RFS, relapse-free survival.

COMBINATORIAL IMMUNOTHERAPY REGIMENS

Cancer vaccines as a single treatment modality are not likely to have the potency required to overcome the obstacles of tumor burden and immune tolerance in patients with established cancer. The scientifically based sequencing of tumor vaccines with surgery, radiation therapy, chemotherapy, and biologically targeted therapies is a critical aspect of clinical tumor vaccine development that should be determined in relevant preclinical models when possible. Traditional drug development typically involves Phase I testing in heavily pretreated patients with extensive disease, a patient population that is not appropriate for testing immunotherapies designed to elicit an effective host antitumor immune response. The detrimental effect of both a greater number of prior chemotherapy regimens and close proximity to a prior chemotherapy treatment on the induction of carcinoembryonic antigen (CEA)-specific T cell precursors in patients with advanced colorectal carcinoma treated with the canary pox vaccine ALVAC-CEA was recently demonstrated.[44] Importantly, the intensity of the vaccine-induced immune response determines tumor clearance.[45] The mismatch between tumor growth kinetics and the intensity of the vaccine-induced antitumor response achievable with current vaccination strategies is a strong argument for testing vaccine therapy in patients with minimal or undetectable residual disease after standard therapy. In a trial design that considers the influence of both tumor burden and aggressive cytotoxic therapy on vaccine-induced immunity, Jaffee and colleagues are conducting a Phase II efficacy trial of an allogeneic GM-CSF-secreting pancreatic cancer vaccine in 60 high-risk pancreatic cancer patients with minimal residual disease after pancreaticoduodenectomy. Patients are vaccinated immediately after surgery (just prior to six months of adjuvant chemoradiation) then go on to receive three additional monthly vaccinations after completing standard therapy. Monitoring the dynamics of antigen-specific vaccine-induced immune responses in these patients should provide further insight into these issues.

Chemotherapeutic agents are commonly used for their overt cytotoxic effects, and at standard doses clearly suppress cellular immune responses. However, some can also either enhance or inhibit antigen-specific immune responses depending on the dose and timing of administration in relation to antigen exposure (reviewed in ref. 46). Sequencing a GM-CSF-secreting CT-26 colon cancer vaccine with low doses of Doxorubicin (2, 4, or 6 mg/kg) one week after vaccination of CT-26 tumor-bearing BALB/C mice results in a higher rate of cure (40%) than either drug (10%) or vaccine (0%) alone.[47] The in vivo synergy was correlated with a dose-dependent increase in antigen-specific cytotoxic T lymphocyte (CTL) activity. Vaccine activity was abrogated when Doxorubicin was given prior to immunization. We investigated interactions between chemotherapy and GM-CSF-secreting vaccine in the tolerogenic HER-2/*neu* breast cancer model.[48] Low doses of Cyclophosphamide (100 mg/kg) or Paclitaxel (20 mg/kg) given one day prior to vaccine significantly decreased the outgrowth of pre-established mammary tumors compared to chemotherapy or vaccine alone. This correlated with the augmentation of HER-2/*neu*-specific IgG titers and HER-2/*neu*-specific T helper type 1 immunity by ELISPOT analysis. The sequence of Cyclophosphamide one day prior to vaccination, immunization, and Doxorubicin (5 mg/kg) seven days after vaccination was most potent, curing up to 20% of the mice. Importantly, both standard doses of the drugs and reversal of the treatment sequence inhibited vaccine activity, again highlighting the importance of dose and timing in relation to antigen exposure. Nowak and colleagues examined the influence of Gemcitabine on the induction of antigen-specific immunity using hemagglutinin (HA) T cell receptor transgenic mice and the HA-expressing mesothelioma cell line AB1-HA.[49] The administration of five doses of Gemcitabine (120 μg/g every three days) to tumor-bearing mice completely abrogated HA-specific IgG responses, with minimal to moderately enhanced HA-specific T cell proliferation. Finally, upregulation of MHC Class I and cancer testis antigens by pretreating tumor cells with the demethylating agent

5-aza-2'-Deoxycytidine in vitro can restore melanoma- and renal cell carcinoma-specific CTL activity.[50,51] Together, these results argue for the careful pharmacodynamic analysis of cancer vaccines and chemotherapeutic agents in clinically relevant preclinical models prior to clinical testing of combinatorial vaccination protocols.

The increasing number of costimulatory molecules that participate in the APC-T cell interaction has created unprecedented opportunities for highly targeted immune manipulation. CTLA4 is a negative regulatory molecule that both attenuates T cell responses and regulates peripheral T cell tolerance.[52] In vivo antibody-mediated blockade of CTLA4 potentiates T cell responses to some (but not all) poorly immunogenic murine tumors,[52] and combining CTLA4 blockade with GM-CSF-secreting vaccines has a synergistic antitumor effect compared to antibody or vaccine alone.[53,54] Further, whereas CTLA4 blockade alone was unable to retard tumor growth in MOPC-315 tumor-bearing mice, combined with low dose Melphalan it had a significant anti-tumor effect.[55] The safety of a fully human CTLA4-specific antibody in patients with prostate cancer or melanoma has been reported, with a suggestion of single agent activity.[52] The CD40/CD154 (CD40L) pathway plays a central role in the regulation of both humoral and cellular immunity.[56] CD40-specific agonist antibodies can substitute for $CD4^+$ T cell help in the priming of $CD4^+$ and $CD8^+$ T cells.[57] These antibodies can both prevent tumor-induced T cell tolerance and break established T cell tolerance, augmenting the efficacy of tumor vaccines in preclinical models.[58,59] OX40 is a third promising costimulatory target for tumor immunotherapy. It is a member of the tumor necrosis factor receptor (TNFR) superfamily expressed transiently on activated $CD4^+$ T cells localized to the site of the immune response.[60] OX40 engagement during primary immunization breaks peripheral $CD4^+$ T cell tolerance[61] and increases the survival of memory T cells by inhibiting AICD.[62] Practically, engagement of the OX-40 receptor in vivo during tumor priming enhances antitumor immunity.[63] Also, combining agonist OX40 antibody and the adoptive transfer of tumor-specific T cells has a greater antitumor effect than T cells alone.[64] These features, together with the correlation of high levels of $OX40^+$ TIL in primary human colon cancers with survival ($p = 0.02$),[65] identify OX40 as a particularly attractive target for drug development. 41BB is a member of the TNFR superfamily that participates in the activation of dendritic cells and T cells.[66] Engagement of the 41BB pathway can eradicate some established tumors.[67] Combining peptide vaccination and 41BB signaling can break immunologic ignorance, resulting in immune-mediated tumor regression.[68] Further dissecting the distinctions between the costimulatory pathways and the potential cross talk between them will enable the development of the most active combinatorial cancer vaccine protocols, as well as their translation to the most appropriate clinical setting.

Lymphopenia-induced homeostatic T cell proliferation is a mechanism for restoring the memory T cell compartment.[69,70] Immune manipulation by active immunization or the adoptive transfer of tumor antigen-specific T cells during the period of immune reconstitution after ablative treatments might favor the development of a T cell repertoire skewed toward a desired antitumor specificity.[71] Consistent with this, the preferential induction and expansion of functional, melanoma-specific T cells in lymphopenic mice with pre-existing tumors vaccinated with a GM-CSF-secreting melanoma vaccine was recently demonstrated to correlate with significant tumor regression.[72] These studies were conducted in Rag-1-deficient mice that are inherently lymphopenic, and it may be argued that this setting is not clinically relevant. However, several other preclinical studies have demonstrated the potential of enhancing antitumor immunity by vaccinating tumor-bearing mice with GM-CSF-secreting tumor vaccines during early engraftment after syngeneic or allogeneic T cell depleted bone marrow transplant (BMT).[73,74] Further, sublethal irradiation of tumor-bearing mice followed by the adoptive transfer of syngeneic T cells can result in an effective antitumor response as measured by CTL activity, IFN-γ secretion, and long-term memory.[75] While the phenomenon of homeostatic T cell proliferation has not yet been demonstrated in humans, lymphopenia is a common result of many standard cancer therapies. Characterization of homeostatic T cell proliferation in cancer patients followed by the careful delineation of the influence of chemotherapy and/or radiation on the kinetics and efficacy of antigen-specific immune reconstitution will be required for the effective application of tumor vaccines to the lymphopenic setting.

ANTIGEN IDENTIFICATION

It is clear that the identification of potent tumor rejection antigens is essential for the development of effective cancer vaccines. Traditional approaches to tumor antigen identification have included genetic strategies that use patient-derived CTL to screen cDNA libraries transfected into cells expressing defined MHC molecules, those that use patient sera to screen tumor-derived cDNA expression libraries (SEREX, or serologic analysis of recombinant cDNA expression), and biochemical strategies that involve the isolation, purification, and sequencing of peptide epitopes bound to MHC pockets.[9] These approaches identified a number of tumor antigens that have already been clinically tested. However, most antigen-specific cancer vaccines tested to date have induced antigen-specific immunity with minimal evidence of a clinically meaningful antitumor immune response.[31-34,37] This is not surprising since the identification of these tumor antigens as targets of T lymphocyte or antibody responses in patients with existing (or progressing) disease decreases the likelihood that they represent true tumor rejection targets. Moreover, traditional strategies for antigen identification cannot identify tumor antigens ignored by the immune system. One way to circumvent these inherent difficulties is to combine genomic databases generated by SAGE and microarray analyses with empiric peptide epitope deduction to identify candidate antigenic epitopes derived from cancer associated genes.[76] These candidate epitopes are tested for their ability to elicit antigen-specific CTL responses from healthy donors and cancer patients ex vivo. This approach has identified telomerase (hTERT)[77,78] and survivin (an anti-apoptotic protein)[79,80] as two candidate universal tumor antigens preferentially expressed by multiple tumor types. Neither hTERT nor survivin has yet been defined as a true tumor rejection target.

While empiric epitope deduction circumvents immunologic ignorance and capitalizes on the power of cancer genomics and bioinformatics, it is limited by the lack of a functional component. Several early trials of either nonspecific immunotherapies ($CD4^+$ donor lymphocyte infusion for relapsed chronic myelogenous leukemia (CML) after BMT),[81] or peptide-based,[82] adenoviral,[83] or GM-CSF-secreting vaccines[11] have demonstrated significant clinical responses in very small numbers of patients. The sera and lymphocytes of these patients represent powerful tools for the identification of tumor antigens for immune monitoring and vaccine development. Patient sera were used to interrogate cDNA expression libraries derived from either CML or melanoma to identify CML66 (a novel gene product)[84] and ATP6S1 (a component of the vacuolar H^+-ATPase complex)[85] as broadly immunogenic tumor antigens expressed by a variety of solid and hematologic tumors. Two other

reports utilized TIL or PBL derived from two responding melanoma patients treated with a multi-peptide vaccine plus interleukin-2 or a MART-1 adenovirus vaccine. These investigators identified the novel, melanoma-specific tumor antigens SOX10 (a transcription factor found in neural crest tissue)[86] and BING-4 (a novel gene product localized to the MHC Class II gene complex).[87] It is interesting to consider that, with the exception of ATP6S1, none of these newly identified candidate tumor antigens were delivered by the immunotherapy. The immune responses thus apparently developed as a result of immune-mediated tumor destruction. Whether responses to these antigens simply represent sentinel markers of effective antitumor immune responses or identify true tumor rejection targets will only be borne out by further study. Regardless, it is clear that combining cancer genomics, bioinformatics, and a detailed immune response analysis of patients who develop clinical responses on cancer vaccine trials is a powerful strategy with the potential for identifying the most biologically relevant tumor antigens. The ultimate goal of such detailed analyses is to identify shared tumor antigens for the formulation of potent recombinant polyvalent cancer vaccines broadly applicable to multiple tumor histologies and MHC haplotypes.

CONCLUSIONS

The field of cancer vaccines has matured significantly since the days of William Coley, largely due to advances in molecular immunology and cancer genetics. Developing strategies for overcoming immune tolerance and identifying the most active tumor rejection antigens are both critical to the success of therapeutic cancer vaccines. The judicious use of clinically relevant preclinical models to identify the most potent vaccination regimens for clinical testing will hasten clinical development. Innovative clinical trial designs, novel trial endpoints,[88] and informative surrogate markers and predictors of clinical response will ensure that cancer vaccines are most effectively incorporated into the management of cancer patients.

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DEVELOPMENT OF A LETHALLY-IRRADIATED GM-CSF-SECRETING ALLOGENEIC BREAST CANCER VACCINE FOR USE IN CLINICAL TRIALS

Leisha A. Emens*, Janice Davis-Sproul, Amy M. Thomas, R. Todd Reilly, Nancy E. Davidson, and Elizabeth M. Jaffee. Johns Hopkins University School of Medicine, Baltimore, MD. *email address: Emensle@jhmi.edu

Immunotherapy with GM-CSF-secreting cancer vaccines incorporating important targets for tumor rejection and immune monitoring is a novel approach to the treatment and prevention of cancer. Early clinical trials of GM-CSF-secreting autologous tumor vaccines quickly demonstrated that it is not technically feasible to expand adequate numbers of autologous tumor cells *in vitro* for vaccine production and immune monitoring. This technical limitation, together with interest in applying this technology to the clinical settings of metastatic disease and prevention (where autologous tumor is typically unavailable), has led us to develop allogeneic GM-CSF-secreting tumor cells as a more generalizeable vaccine platform for delivering shared tumor antigens. We are now applying this strategy to breast cancer, and have developed a lethally irradiated GM-CSF-secreting allogeneic breast cancer vaccine for use in clinical trials. After screening five human breast cancer cell lines for the expression of proteins known to be important in breast cancer immunobiology, we chose MCF7, SKBR3, and T47D for vaccine development. These lines were chosen to ensure the delivery of a balanced repertoire of known tumor antigens, including HER-2/*neu*, mucin-1, MAGE1, MAGE3, CEA, and p53. These cell lines were genetically modified by plasmid DNA transfection to generate subclones secreting high levels of human GM-CSF. 2MCF7-1, 3SKBR3-7, and 2T47DV produce GM-CSF at levels of 325, 60, and 325 ng/10^6cells/24 hours respectively as determined by GM-CSF ELISA and confirmed by TF-1 bioassay. We have designed the vaccine to deliver the breast tumor antigen HER-2/*neu*, thus facilitating *in vivo* monitoring of vaccine-activated immunity by assessing delayed type hypersensitivity (DTH) to known HER-2/*neu* T helper peptide epitopes. The vaccine will be formulated from 3SKBR3-7 and 2T47DV, both of which express HER-2/*neu* and are wild type for k-*ras* sequences by PCR analysis. 2MCF7-1 was excluded since it contains sequences corresponding to the mutated k-*ras* T helper peptide epitope that will be used as a negative control for the *in vivo* immune monitoring of DTH to HER-2/*neu*. Further studies established the doses of radiation required to induce apoptosis yet maintain GM-CSF-secretion for the requisite 4 to 5 days as 10,000 rads for 3SKBR3-7 and 20,000 rads for 2T47DV. The vaccine cells are lethally irradiated, then frozen in a directly injectable cryoprotectant consisting of 70% plasmalyte-A, 20% human serum albumin (V/V), and 10% DMSO in order to minimize the manipulation required on the day of inoculation. We have thus developed a broadly applicable GM-CSF-secreting human breast cancer vaccine capable of delivering a variety of known (and unknown) tumor antigens, and incorporating HER-2/*neu* as a target for the monitoring of vaccine-activated immunity in clinical trials.

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Combined Passive (Monoclonal Antibody Infusion) and Active (Whole-cell Vaccination) Immunotherapy is More Effective than Either Modality Alone in the Eradication of HER-2/*neu*-Expressing Mammary Tumors

Matthew Wolpoe, Eric Lutz, Anne Ercolini, Mark Greene, Leisha Emens, Elizabeth Jaffee, and R. Todd Reilly. The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, Maryland.

HER-2/*neu* is a proto-oncogene implicated in several cancers including approximately 30% of breast carcinomas and is an independent risk factor for decreased survival and distant metastases. HER-2/*neu* transgenic mice that overexpress the non-transforming rat proto-oncogene (neu-N mice) develop spontaneous mammary carcinoma beginning at approximately 5 months of age that are histologically similar to those of human breast cancer. In addition, neu-N mice demonstrate tolerance to neu-expressing mammary tumors that resembles that seen in cancer patients. Parental (nontolerogenic) FVB/N mice mount significant cellular and humoral immune responses to neu-specific vaccination and are fully protected from neu-expressing tumor (NT) challenge after neu-specific whole-cell vaccination. In contrast, neu-N mice mount a measurable, albeit weaker, cellular response to neu-specific whole-cell vaccination with little or no induction of neu-specific IgG, resulting in delayed tumor growth but essentially no cures. Using an adoptive transfer model for immune-mediated tumor rejection, we demonstrated a requirement for both humoral and cellular immunity to neu to fully eradicate an established NT burden. Because monoclonal antibody therapy with Herceptin for neu-expressing tumors plays a vital role in the clinical management of breast cancer, we sought to determine whether the passive infusion of neu-specific monoclonal antibody (mAb), effectively reconstituting the humoral arm of the neu-specific immune response, combined with vaccine-induced neu-specific cellular immunity resulted in more potent antitumor immunity in this pre-clinical model system. The combination of a single neu-specific mAb with neu-specific whole-cell vaccine resulted in rejection of an NT challenge by 20-40% of neu-N mice, depending on the antibody used. In contrast, vaccination or mAb treatment alone yielded only delayed tumor growth. Notably, when a combination of two mAbs was used with vaccine, tumor free survival approached 70%. Similar efficacy was seen in experiments designed to eradicate an established NT burden, with 40% of mice treated with a combination of both antibodies and vaccine remaining tumor free. ELISPOT assays show an increase in neu-specific CD8+ cells in neu-N mice treated with neu-specific mAbs plus vaccine compared to either whole cell vaccine or antibody alone. These data demonstrate that active immunotherapy with a neu-specific whole-cell vaccine combined with passive immunotherapy using neu-specific mAbs leads to a synergistic anti-tumor response and a significant improvement in tumor-free survival in a tolerized host.

Reversal of CD8+ Peripheral Tolerance in the HER-2/neu Transgenic Mice by Deletion of $CD4^+CD25^+$ Regulatory T Cells

Anne M. Ercolini, Brian Ladle, Todd D. Armstrong, Jean-Pascal H. Machiels, Leisha A. Emens, R.Todd Reilly, and Elizabeth M. Jaffee, Department of Oncology, Johns Hopkins University, Baltimore, Maryland 21231, USA

Central and peripheral mechanisms of T cell tolerance must exist to prevent autoimmunity against normal or self-antigens. These same mechanisms that establish and maintain self-tolerance are likely contributors to the unresponsive state of T cells often observed in cancer patients. $CD8^+$ T cells specific for tumor antigens have been detected in patients. These T cell responses are usually observed in patients who have been treated with an antigen-specific vaccine. In the majority of these patients, the T cell responses are weak and ineffective at controlling tumor growth. In some instances, this may be due to an ineffective vaccine approach. However, in may cases, mechanisms of T cell tolerance to specific tumor antigens have also been implicated. Intrathymic expression of self-antigens often leads to central deletion of T cells that express high avidity T cell receptors (TCR) specific for these antigens. However, T cells that express lower avidity TCRs for these antigens can escape into the periphery. In addition, both high avidity and low avidity T cells specific for peripherally expressed self-antigens are expected to be positively selected in the thymus rather than undergo deletion. Therefore, peripheral (extrathymic) mechanisms exist that either render the T cell ignorant or functionally impaired to self-antigens in tissues. Evidence suggests that these mechanisms of peripheral tolerance are biologically important for suppressing autoreactive T cells. However, understanding these mechanisms in the context of tumor antigens should lead to the development of interventions that can reverse the tolerant state and allow T cells to more effectively respond to tumors. Her-2/neu transgenic mice overexpress the wild type rat proto-oncogene neu and develop spontaneous mammary tumors between 4 and 8 months of age. These mice exhibit down-regulated T and B cell responses that are unresponsive to neu-targeted vaccination. This ability to detect neu-specific T cells in the setting of actively growing neu-expressing tumors, is a situation similar to that observed in breast and ovarian cancer patients whose tumors overexpress HER-2/neu. In contrast, these same neu targeted vaccines induce vigorous antitumor immunity that can eradicate large burdens of neu expressing tumors in the parental non-tolerized mice. We have recently identified a 10 amino acid epitope, $RNEU_{420-429}$, as the immunodominant epitope against which all neu vaccine induced T cells are directed in the parental non-tolerized mice. In contrast, neu vaccine induced T cells isolated from the neu transgenic mice are rarely directed against this epitope. When present, $RNEU_{420-429}$ T cells are of low avidity and do not eradicate neu expressing tumors. However, high avidity $RNEU_{420-429}$ specific T cells can be recovered in tumor bearing mice that are treated with immune modulating agents in combination with a neu targeted vaccine. The presence of these high avidity T cells is associated with tumor rejection. The mechanism by which these high avidity T cells are recovered will be discussed. NIH/NCI NCDDG Grant 2U19CA72108 (EMJ), SPORE in Breast Cancer 1P50CA88843 (EMJ), NIH/NIAID Grant 5T32AI07247-21 (AME), DAMD17-01-1-0282 (AME), CRI Grant 311-2007 (AME), and the Howard Hughes Medical Institute Medical Student Research Training Fellowship (BL).

317 MHC class II cytoplasmic domains regulate class II localization to lipid rafts and activation of tumor-specific CD4+ T cells.

Brian P. Dolan[1], Timothy P. Phelan[1], Dan Ilkovitch[1], Ling Qi[1], Terri M. Laufer[2], William F. Wade[3], Suzanne Ostrand-Rosenberg[1]

[1]Department of Biological Sciences, University of Maryland Baltimore County, Baltimore, MD 21250

[2]Division of Rheumatology, University of Pennsylvania 421 Curie Blvd., BRB II/III Rm 753, Philadelphia, PA 19104

[3]Department of Microbiology and Immunology, Dartmouth Medical School, Lebanon, NH 03756

Cell-based tumor vaccines consisting of parental tumor cells engineered to express MHC class II molecules stimulate tumor-specific CD4+ T cells to mediate rejection of established poorly immunogenic tumors. Previous experiments have demonstrated that these vaccines induce immunity by functioning as antigen presenting cells for tumor-encoded antigens. However, deletion of the MHC class II cytoplasmic domain abrogates immunogenicity of such vaccines. Recent reports highlighted the role of lipid microdomains in antigen presentation. To determine if truncation of MHC class II molecules impacts localization to lipid rafts, we examined the lipid raft affinity of cytoplasmic domain deleted MHC class II molecules in B cell lymphomas and mouse spindle cell sarcoma vaccine cells. In both cell types, truncation of either the alpha or beta chain of the MHC class II heterodimer decreased the affinity of class II molecules for lipid rafts, but did not abolish raft localization. Simultaneous deletion of both cytoplasmic domains significantly reduces localization of class II to lipid rafts. Previous in vivo tumor rejection studies demonstrated that cell-based vaccines containing one full length and one truncated chain are sufficient to facilitate tumor rejection. Data reported here suggest that a threshold level of MHC class II localized to lipid rafts is sufficient for successful in vivo antigen presentation. In vitro antigen presentation assays demonstrate that disruption of lipid rafts by cholesterol depletion inhibits antigen presentation by B cell lymphoma cells containing one or two full-length MHC class II chains. In contrast, cholesterol depletion has minimal impact on APC with both cytoplasmic domains deleted. Collectively, these data suggest that the cytoplasmic domains of MHC class II molecules are important for lipid raft localization which, in turn, enhances antigen presentation and vaccine efficacy of MHC class II-expressing tumor cell vaccines. (NIH CA52527, NIH CA84232, DAMD-17-01-1-0312)

318 An alternate, lower-affinity neu-specific T cell repertoire in HER-2/neu transgenic mice relative to the parental strain may explain neu-specific tolerance to neu-expressing tumors

Ercolini, A.M., Armstrong, T., Ladle, B., Machiels, J.P., Lei, R., Reilly, R.T and Jaffee, E.M. Department of Oncology, The Johns Hopkins University School of Medicine, Baltimore, MD 21231.

The proto-oncogene HER-2/neu (*neu*) is overexpressed by 30-40% of all breast cancers. Although patients with neu expressing tumors develop antibody and T cell responses to this antigen, these responses are weak and unable to hinder tumor growth. This suggests that T cell tolerance is a significant barrier to immune based treatments that target neu in breast cancers. The *neu*-N transgenic mice developed by Muller from the FVB/N parental strain overexpress the non-mutated rat *neu* cDNA under the MMTV promoter. These mice develop spontaneous mammary tumors beginning at 4 months of age. Data strongly supports the existence of tolerance in these mice. We have therefore isolated $CD8^+$ T cell clones and lines from both the parental and transgenic mice following vaccination. We identified $RNEU_{420-429}$ as the immunodominant epitope in rat neu based on the fact that numerous T cell lines and clones developed from vaccinated FVB/N mice recognized this peptide. MHC/peptide tetramer analysis shows that neu-targeted vaccination induces high-avidity $RNEU_{420-429}$ specific T cell activity in the FVB/N mice. In contrast, neu-targeted vaccination of *neu*-N mice demonstrates some lower avidity $RNEU_{420-429}$ activity. Importantly, treatment of *neu*-N mice with immunomodulating doses of chemotherapy along with vaccination will uncover high avidity $RNEU_{420-429}$ specific $CD8^+$ T cell activity and is associated with more effective eradication of neu-expressing tumor *in vivo*. To further characterize the response to neu in FVB/N and *neu*-N strains, mice were given neu-expressing vaccine either alone or in combination with chemotherapy. Whereas all FVB/N mice developed CTL specific for $RNEU_{420-429}$, only 20% of *neu*-N mice given chemotherapy along with vaccine developed T cells specific for the immunodominant peptide. Although the response to neu was in general poor as compared with the FVB/N response, several *neu*-N mice developed T cells that were reactive to the full-length neu protein but not to its immunodominant peptide. These data suggest that tolerance mechanisms in *neu*-N mice appears to affect mostly high-avidity T cells specific for the immunodominant epitope in neu, leaving in place cells that are low avidity, cells that are specific for a less immunogenic cryptic epitope, or perhaps both. However, this tolerance may be overcome with the proper vaccination regimen.
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319 All-trans-retinoic acid eliminates immature myeloid cells from tumor-bearing mice and improves the effect of vaccination

Dmitry Gabrilovich, Sergey Kusmartsev, Fengdong Cheng, Bin Yu, Yulia Nefedova, Eduardo Sotomayor, Richard Lush, *H.Lee Moffitt Cancer Center, University of South Florida, Tampa, FL, 33612*

Tumor-induced immunosuppression is one of the crucial mechanisms of tumor evasion of immune surveillance. It contributes greatly to the failure of cancer vaccines. Immature myeloid cells (ImC) play an important role in tumor-induced immunosuppression. These cells accumulate in large numbers in tumor-bearing hosts and directly inhibit T-cell functions via various mechanisms. These cells can inhibit the very same antigen-specific immune response that a cancer vaccine is trying to generate. All these data suggest that elimination of ImCs may significantly improve antitumor immune response and enhance the effect of cancer vaccines. One of the approaches to achieve this goal could be the differentiation of ImCs. In our previous studies *in vitro* we have demonstrated that several combinations of growth factors that promote differentiation of dendritic cells, macrophages and granulocytes provided a rather minor effect on population of ImCs. At the same time, all-trans-retinoic acid ATRA dramatically reduced the presence of ImCs. In this study we tried to eliminate ImCs in an attempt to improve antitumor response. *In vivo* administration of ATRA dramatically reduced the presence of ImCs in all three tested tumor models. This effect was not due to a direct antitumor effect of ATRA or decreased production of growth factors by tumor cells. Experiments with adoptive transfer demonstrated that ATRA differentiated ImCs *in vivo* into mature DCs, macrophages and granulocytes. Decreased presence of ImCs in tumor-bearing mice reversed tumor induced $CD4^+$ T cell tolerance and noticeably improved CD8 mediated tumor-specific immune response. Combination of ATRA with 2 different types of cancer vaccines (peptide in CFA and dendritic cells transduced with wild-type p53) in two different tumor models (C3 tumor and MethA sarcoma) significantly prolonged the antitumor effect of the treatment. These data suggest that elimination of ImCs with ATRA may open an opportunity to improve the effect of cancer vaccines.

320 Tumour Immunity Induced by Immunisation with B16FasL in the Absence of $CD25^+$ Regulatory Cells

Anna Katharina Simon*, Emma Jones^, Gavin Screaton* and Awen Gallimore@

* Institute of molecular Medicine, John Radcliffe Hospital, Oxford.

^ Nuffield Department of Medicine, John Radcliffe Hospital, Oxford.

@ Medical Biochemistry, University of Wales College of Medicine, Cardiff.

Using a murine model, we have found that induction of immune responses capable of controlling growth of the melanoma cell-line B16, can be achieved in different ways. Firstly, removal of $CD25^+$ regulatory cells *in vivo* can result in tumour rejection following inoculation of mice with live B16 cells. In this case, immunity is mediated by $CD4^+$ T cells. Secondly, we have found that tumour immunity can be generated by inoculating mice with B16 cells engineered to express FasL. In this case, immunity is mediated by antibodies. In our experimental model, depletion of $CD25^+$ regulatory cells or immunization of mice with B16FasL typically induces long-term immunity to unmanipulated B16 in approximately 50% of mice. Several independent experiments have, however, shown that long-term immunity can be achieved in at least 90% of mice immunized with B16FasL in the absence of $CD25^+$ regulatory cells. The superior protection against tumour growth obtained using the latter protocol could reflect induction of both an antibody response and a $CD4^+$ T cell response capable of rejecting B16. Alternatively, depletion of $CD25^+$ regulatory cells may enhance the antibody response induced by B16FasL. These possibilities are currently under investigation.
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